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MICROBIALLY MEDIATED REDUCTIVE DECHLORINATION OF DICHLOROBENZENE

A Thesis
Presented to the Faculty of the Graduate School
of Cornell University
In Partial Fulfillment of the Requirements for the Degree of
Master of Science

by
Anne Sandra Quistorff
August 1999

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The microbially mediated reductive dechlorination of 1,2-, 1,3-, and 1,4-dichlorobenzene (DCB) was studied using site material from eight different locations. The soil and groundwater from each site were anaerobically added to multiple serum bottles and mixed. All three DCB isomers were added to each serum bottle to a total nominal concentration of 30 micro-mole per liter for some microcosms and 600 micro-mole per liter for other microcosms. In addition, electron donor in the form of yeast extract (100 mg/L) was added to some microcosms and some microcosms were autoclaved and served as abiotic controls. The microcosms were then incubated, inverted, in the dark at 24 deg C and periodically monitored by headspace injections into a gas chromatograph system. Reductive dechlorination of the DCB isomers was noted in microcosms from five of the eight locations and ranged from 10% to nearly 100%. Additionally, in all the positive microcosms, dechlorination proceeded independent of whether yeast extract was added. Due to the rapid onset of dechlorination in some of the microcosms, it is likely that DCB dechlorination is occurring on site. Generally, 1,2-DCB was the nost readily degraded DCB isomer. 1,3-DCB was the next most readily degraded isomer, distantly followed by 1,4-DCB.

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MICROBIALLY MEDIATED REDUCTIVE DECHLORINATION OF DICHLOROBENZENE

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ABSTRACT

The microbially mediated reductive dechlorination of 1,2-, 1,3- and 1,4-dichlorobenzene (DCB) was studied using site material from eight different locations: Plattsburgh Air Force Base (AFB), Kelly AFB, four different locations at Robins AFB, Louisiana wetland sediment, and digested sludge from the Ithaca area wastewater treatment plant. The soil and groundwater from each site were anaerobically added to multiple serum bottles and mixed. All three DCB isomers were added to each serum bottle to a total nominal concentration of 30 μmole/L for some microcosms and 600 μmole/L for other microcosms. In addition, electron donor in the form of yeast extract (100 mg/L) was added to some microcosms and some microcosms were autoclaved and served as abiotic controls. The microcosms were then incubated, inverted, in the dark at 24°C and periodically monitored by headspace injections into a gas chromatograph system.

Biologically mediated transformation of DCBs was determined by noting a decrease in DCB levels and a corresponding increase in monochlorobenzene (MCB) levels in active microcosms while noting no change in the autoclaved controls.

Reductive dechlorination of the DCB isomers was noted in microcosms from five of the eight locations: Louisiana wetland sediment, Kelly AFB, Robins AFB at well BIA4 at both 17- and 25-foot depths and slight dechlorination for the digested sludge. The extent of MCB production from DCB dechlorination was about 10%

for the digested sludge and ranged from about 50% to 100% for the other microcosms. The most successful microcosms (exhibiting close to 100% transformation in some instances) were the Louisiana wetland sediment and the Robins AFB well BIA4 at 25-foot depth.

Additionally, in all the positive microcosms, dechlorination proceeded independent of whether yeast extract was added -- probably because the indigenous levels of electron donor were sufficient for dechlorination. Due to the rapid onset of dechlorination in some of the microcosms prepared from Robins AFB material, it is likely that DCB dechlorination is occurring on site. From all these sites (except digested sludge) successful enrichment cultures in basal medium have been developed.

Generally, 1,2-DCB was the most readily degraded DCB isomer. 1,3-DCB was the next most readily degraded isomer, distantly followed by 1,4-DCB.

Slight benzene production from DCB dechlorination was observed in microcosms prepared from material from Robins AFB at well BIA4. Benzene production only seemed to occur while DCBs were being dechlorinated, even when MCB was otherwise at high concentrations. The benzene production accounted for about 1% of the total recovered MCB. The low levels of benzene observed appear to be caused by co-metabolic transformation coincident to DCB dechlorination.

BIOGRAPHICAL SKETCH

Anne Sandra Quistorff was born to Helen Josephine Quistorff and Kirk Dean Quistorff on July 27, 1975. She grew up on Bainbridge Island Washington, and attended Bainbridge High School. After high school she attended Cornell University and graduated with a BS in Civil and Environmental Engineering in May of 1997. In June of 1997 Anne started work on her Masters Degree at Cornell University.

This thesis is dedicated to my parents,

Kirk and Helen Quistorff.

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LIST OF ABBREVIATIONS

AFB	air force base
BESA	2-bromoethanesulfonic acid
BTEX	benzene, toluene, ethylbenzene and xylene
Cd	cadmium
CH_4	methane
CO_2	carbon dioxide
DCB	dichlorobenzene
DS	digested sludge from the Ithaca waste water treatment plant
ECD	electron capture detector
EPA	environmental protection agency
FID	flame-ionization detector
FPD	flame-photometric detector
GC	gas chromatography
GC/MS	gas chromatography / mass spectrometry
H_2	hydrogen
HCB	hexachlorobenzene
K	Kelly Air Force Base
L	Louisiana wetland sediment
LNAPL	light non-aqueous phase liquid
MCB	monochlorobenzene
MCL	maximum contaminant limit
MS	molecular sieve
N_2	nitrogen
O_2	oxygen
P	Plattsburgh Air Force Base
Pb	lead
PeCB	pentachlorobenzene
R-1-d	Robins Air Force Base well BIA4 at 25 feet
R-1-s	Robins Air Force Base well BIA4 at 17 feet
R-2	Robins Air Force Base well R13-2W
R-LF	Robins Air Force Base landfill leachate
RGD	reduction-gas detector
TCB	trichlorobenzene
TCD	thermal-conductivity detector
TCE	trichloroethene
TeCB	tetrachlorobenzene
VFA	volatile fatty acid
YE	yeast extract

CHAPTER ONE -- INTRODUCTION

1.A Context

Dichlorobenzenes (DCBs) are common ingredients in such items as deodorizers, solvents, pesticides, herbicides and cleaners. Through their use they have become pollutants in surface waters, sewage and groundwater. The Environmental Protection Agency (EPA) estimated that between 1987 and 1993, 75,967 pounds of 1,2-DCB and 33,675 pounds of 1,4-DCB were released to the water (Environmental Protection Agency, 1998b; Environmental Protection Agency, 1998c). Since 1,2- and 1,4-DCB are widely used, the EPA regulates their concentrations in drinking water. The maximum contaminant level (MCL) for 1,2-DCB is 0.6 mg/L and the MCL for 1,4-DCB is 0.075 mg/L (Environmental Protection Agency, 1998b; Environmental Protection Agency, 1998c).

The EPA has found that long term exposure to 1,2-DCB above the MCL has the potential to cause damage to the liver, kidneys, and nervous system. Long term exposure to 1,4-DCB above the MCL can cause anemia, skin lesions, and atrophy of the liver. Acute exposure to 1,4-DCB can cause vomiting, headaches and irritation of the eyes. In addition, 1,4-DCB is a suspected carcinogen (Environmental Protection Agency, 1998b; Environmental Protection Agency, 1998c).

Currently there are around 300,000 - 400,000 sites at which the groundwater may be contaminated by toxic chemicals (National Research Council, 1994). Russell et al. estimated that it would cost the country between 480 billion to one trillion dollars to clean up all

the groundwater contamination using standard technology (Russell et al., 1991).

The pump-and-treat method is the standard means to clean contaminated groundwater. This method operates by pumping groundwater to the surface, cleaning it and either pumping it back into the soil or disposing of it. Not only is this method expensive but not very efficient. In one site in New Jersey, a company spent 10 million dollars cleaning the site by pump-and-treat until the water appeared to meet the standards. After the pumps were turned off, the pollutant concentration increased to levels higher than before the pumping commenced (National Research Council, 1994). Out of 77 contaminated sites in a National Research Council study, only eight sites had been cleaned to acceptable levels (National Research Council, 1997). The pump-and-treat method fails in many cases for several reasons: immiscibility of certain contaminants in water, the diffusion of contaminants into micropores where water does not easily reach, the sorption of contaminants to subsurface materials and also the heterogeneity of the subsurface resulting in heterogeneous flow patterns (National Research Council, 1997). All these problems can retard contaminant removal and thus prolong the length of time necessary to clean a site. For these reasons, researchers have sought alternatives to pump-and-treat technology.

One class of alternatives to conventional pump-and-treat is in situ bioremediation. This technology involves allowing the native microbes in the groundwater system to degrade the contaminants. Although this remediation alternative is less utilized than

conventional systems, it has several advantages. First, the contaminants are treated in the soil and do not have to be treated or disposed after removal. Second, due to savings in pumping and treating costs and lack of need for disposing of the contaminated groundwater, this alternative is often less costly. Finally, bioremediation may be faster at cleaning than pump-and-treat (National Research Council, 1994).

Within the class of in situ bioremediation there are two main technologies. The first -- enhanced bioremediation -- involves pumping nutrients such as nitrogen and phosphorus, electron acceptors such as O_2, NO_3^{-1} and SO_4^{-2} or electron donors such as H_2 sources, acetate or formate into the contaminated system. The second technology, called intrinsic remediation or natural-attenuation, involves no human intervention in the biological activities. The natural bacteria use the electron acceptors and donors and nutrients readily available at the site to decontaminate the pollutant. This technology does involve extensive monitoring of the plume for such factors as O_2, pH and contaminant concentration to insure that the bacteria are still degrading the contaminant and that the plume is not spreading (National Research Council, 1994).

In situ aerobic degradation of DCBs by bacteria native to contaminated sites has been documented (Kuhn et al., 1985). Several reports have also documented the subsequent isolation of the microbes responsible for the aerobic degradation of DCBs (deBont et al., 1986; Schraa et al., 1986; van der Meer et al., 1987; Spain and Nishino, 1987; Haigler et al. 1988; Oltmanns et al., 1988;

Brunsbach and Reineke, 1994; Spiess et al., 1995; Ravatn et al., 1998). Anaerobic degradation of DCBs are not as well studied. A few reports document more-chlorinated benzenes (hexachlorobenzene, pentachlorobenzene, tetrachlorobenzene or trichlorobenzene) being dechlorinated to DCBs (Bosma et al., 1988; Fathepure et al., 1988; Holliger et al., 1992; Adrian et al., 1997; Susarla et al., 1997; Jackson and Pardue, 1998; Chang et al., 1998). Fewer studies have found DCBs being dechlorinated to monochlorobenzene (MCB) (Ramanand et al., 1993; Masunaga et al., 1996; Middeldorp et al., 1997). Finally, one report documented the transformation of slight amounts of MCB to benzene (Nowak et al., 1996) under anaerobic conditions. Learning more about the possibility for anaerobic degradation of DCBs and the fate of the degradation byproducts is important because many contaminated sites are anaerobic. This knowledge will be essential for determining whether DCBs can be naturally attenuated at anaerobic sites.

1.B Experimental Objectives

The objective of this experiment was to investigate the potential for anaerobic transformations of DCBs. The experiment was conducted by first obtaining soil and nearby groundwater from contaminated sites. The soil and groundwater from each site were then anaerobically added to multiple serum bottles and mixed. All three DCB isomers were added to each serum bottle. In addition, electron donor in the form of yeast extract was added to some bottles and some bottles were autoclaved and served as abiotic

controls. The bottles were then incubated inverted in the dark at 24°C and periodically monitored by headspace injections into a gas chromatograph system. Biologically mediated transformation of DCBs would be determined by noting a decrease in DCB levels and a corresponding increase in MCB levels in active bottles while noting no change in the autoclaved controls.

CHAPTER TWO -- BACKGROUND

2.A Field Evidence for In Situ Degradation of DCBs

Due to the advantages of in situ bioremediation, there have been several studies conducted on the extent of DCB contamination and the possibility of using in situ bioremediation as a clean-up alternative. One study, conducted by Oliver et al. in 1982, took surficial sediment, water, fish and sediment core samples from the lakes Superior, Huron, Erie and Ontario. All lake, river, wastewater effluent and drinking water DCB concentrations were in the ng/L range (well below the MCL, see Section 1.1), with 1,4-DCB as the most prevalent contaminant. The surficial sediment and fish samples showed DCB contamination on the order of 1 mg DCB per g soil. In addition, stratification within the sediment cores allowed Oliver et al. to conclude that most of the DCBs entered the system after the early 1940's. Oliver et al. believed that since the ratios of DCBs to higher-chlorinated benzenes hadn't changed over time, there was no degradation of the DCBs occurring in situ (Oliver et al., 1982). This conclusion was disputed by Bailey who believed that the data presented by Oliver et al. allowed for the possibility of degradation of the lower-chlorinated benzenes (Bailey, 1983).

In 1985, Kuhn et al. observed that DCB contamination was rapidly removed during the infiltration of river water to groundwater from the River Glatt and the River Aare in Switzerland (Schwarzenbach et al., 1983). They postulated that this removal was occurring due to biological processes and thus constructed columns

packed with the river sediment to study this process. Artificial river water with the same pH as the natural site was continuously passed though the column in an upflow manner. From this study they found that, as in the field, DCBs were removed under aerobic conditions but not under anaerobic conditions. In addition, the modeled rates of DCB degradation at the beginning of the aerobic portion of the experiment (0.5 - 1 day^{-1}) were very similar to that observed at the site (~0.4 day^{-1}) (Kuhn et al., 1985).

In contrast, another study done in 1988 by Barber et al. found little transformation of DCBs in situ. The site was Otis Air Force Base, which had been contaminated by disposal of secondary treatment effluent since 1936. They measured 670 ng/L for 1,2-DCB, 600 ng/L for 1,4-DCB, 30 ng/L for 1,3-DCB and 20 ng/L for monochlorobenzene (MCB). These concentrations are low and well below the MCL. They believed that since the concentration of DCBs in the downgradient portion of the plume were equal or greater than the present-day effluent, no degradation of the DCBs had occurred. They inferred that no oxidative degradation occurred because all the dissolved organic carbon (DOC) was utilized in the anaerobic portion of the plume (Barber et al., 1988). In addition, since DCBs were introduced at the same time as branched-chain alkylbenzenesulfonic acids, that these nonbiologically degradable surfactants might be hindering the growth of bacteria (Barber, 1988).

In 1990, Wilson et al. studied the removal of BTEX and MCB at the U. S. Coast Guard Air Station in Grand Traverse County, Michigan. Most of their study was focused on the BTEX but they did

find an order of magnitude decrease in MCB in both the aerobic and anaerobic microcosms created from that site. They found a 44% decrease in autoclaved controls, most likely due to sorption (Wilson et al., 1990).

In 1992, Acton et al. constructed anaerobic columns from aquifer material in contaminated landfill sites from Ontario. They found no degradation of MCB under sulfate-reducing, denitrifying or methanogenic conditions (Acton and Barber, 1992).

In 1993, Beurskens et al. studied the biodegradability of hexachlorobenzene (HCB) contamination in the sediment of the Rhine river. They compared sediment samples taken and frozen in 1972 with slices of sediment cores deposited in the 1970s and taken in 1988 from the same location. They found lower levels of HCB in the sediment core deposited in the 1970s than in the frozen sample from 1972. Conversely, they found higher levels of DCBs in the sediment core deposited in the 1970s than in the frozen sample from 1972 (Table 2.1). These differences the authors believed were due to in situ biodegradation of HCB to DCB (Beurskens et al., 1993).

Table 2.1 -- Differences between HCB and DCB concentrations in sediment samples taken in 1972 and sediment cores deposited around 1970 and taken in 1988.

	Mean concentration nmol/kg		
	Sediment sample taken in 1972	Sediment core deposited around 1970	Difference
HCB	606	121	-485
1,2-DCB	1184	2013	+832
1,3-DCB	350	2388	+2038
1,4-DCB	2759	3608	ns

ns = not significant

2.B Aerobic Degradation of DCBs

Bacterially mediated aerobic degradation of all three DCB isomers is well-documented. It was reported as early as 1986 by deBont et al. They incubated soil and water samples in a stoppered flask while adding 1,3-DCB to the vapor phase. The flask was opened once a week to allow O_2 to enter the system. After six months they found an organism that could degrade 1,3-DCB based on the fact that the pH of the medium dropped below five due to chloride ion release. This organism was tentatively identified as a species of *Alcaligenes*. They further demonstrated that this organism was growing on 1,3-DCB because of the linear relationship between both chloride ion release and protein and 1,3-DCB concentration. The doubling time of this organism growing on 1,3-DCB was 15-hours, which was long compared to its eight-hour doubling time on glucose. This result might suggest that the growth of this organism on 1,3-DCB contained a step that was growth-rate-limiting. The metabolic route of the DCB degradation was studied by measuring O_2 uptake rates when grown on postulated intermediates and measuring postulated intermediate enzyme activity. They proposed a pathway for DCB degradation similar to benzene oxidation (Figure 2.1). This pathway involves a dioxygenase attacking the benzene ring and adding hydroxyl groups in an ortho configuration (deBont et al., 1986).

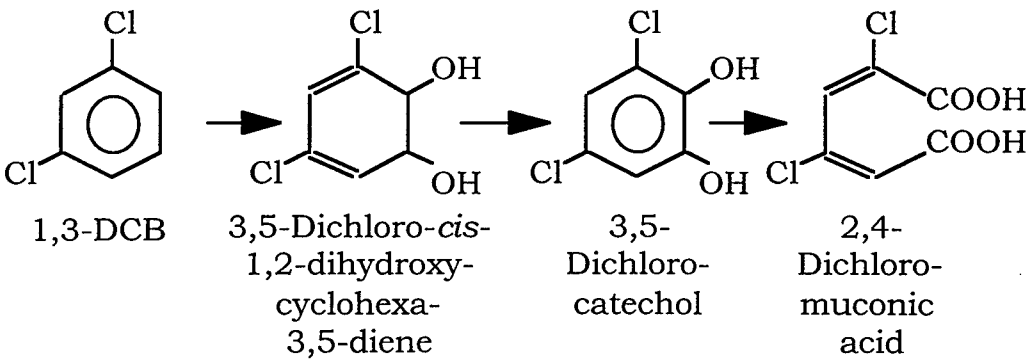


Figure 2.1 -- Proposed pathway for 1,3-DCB oxidation by deBont et al.

Also in 1986, Schraa et al. identified a bacterium that could oxidize 1,4-DCB. After two months of incubation, 1,4-DCB degradation was observed. However it took another 10 months before two different bacteria growing on 1,4-DCB could be isolated. Both these organisms lost their ability to grow on 1,4-DCB after five transfers in media without 1,4-DCB. One of the strains was tentatively identified as a *Alcaligenes* species. It exhibited a linear relationship between cell growth (expressed as protein content) and DCB concentration. They also concluded that the optimum temperature for this organism was 29°C. The metabolic pathway was determined by a similar method to deBont et al., and the pathway for DCB oxidation was very similar to deBont et al. (first three steps of Figure 2.2) (Schraa 1986).

In 1987, Spain et al. found a bacterium tentatively identified as a *Pseudomonas* species, capable of oxidizing 1,4-DCB. They ran tests with radiolabeled 1,4-DCB and found that a considerable fraction of

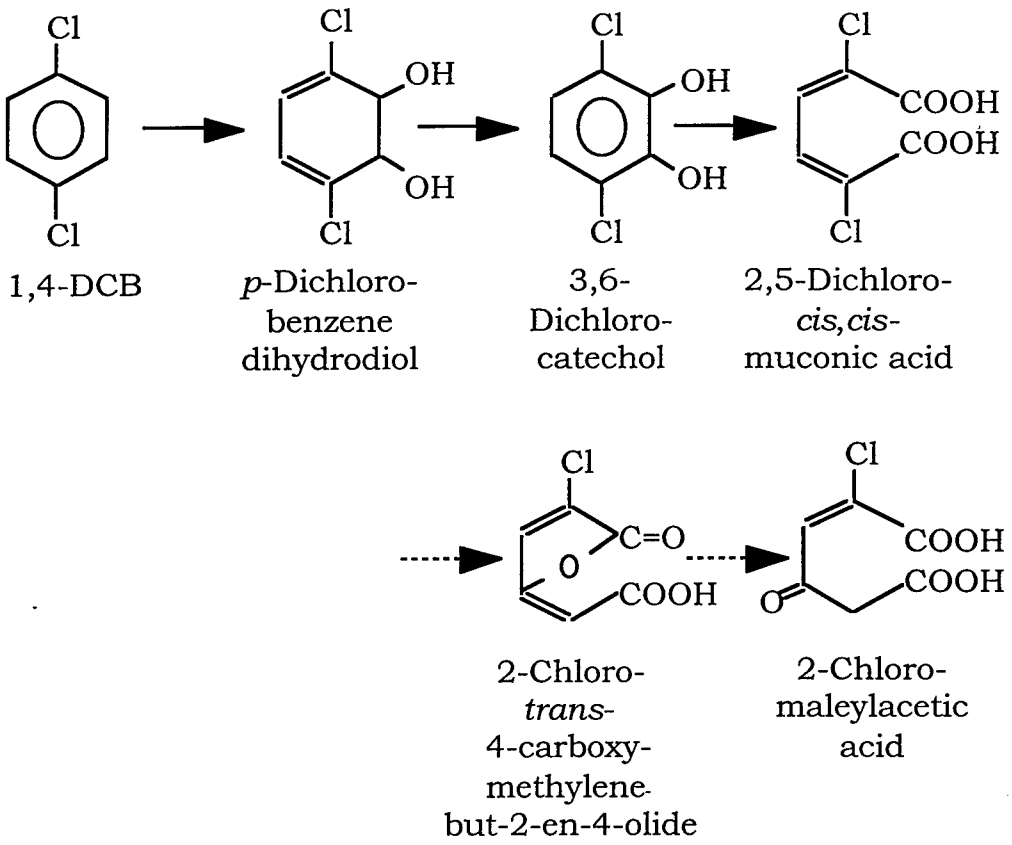


Figure 2.2 -- Proposed pathway for 1,4-DCB oxidation by Schraa et al. (through 2,5-Dichloro-*cis*-*cis*-muconic acid) and Spain et al. Dashed arrows indicate reactions of less certainty.

the carbon from 1,4-DCB was incorporated into the cell material. The first three steps of Spain et al.'s pathway are identical to Schraa et al.'s pathway. Due to the appearance of a compound with spectral properties similar to 2-chloromaleylacetic acid, they tentatively expanded the pathway by two steps (Figure 2.2) (Spain et al., 1987).

In 1988, Haigler et al. isolated a bacterium capable of oxidizing 1,2-DCB from sewage samples collected at Tyndall Air Force Base. Degradation of 1,2-DCB was detected after 14 months. The organism

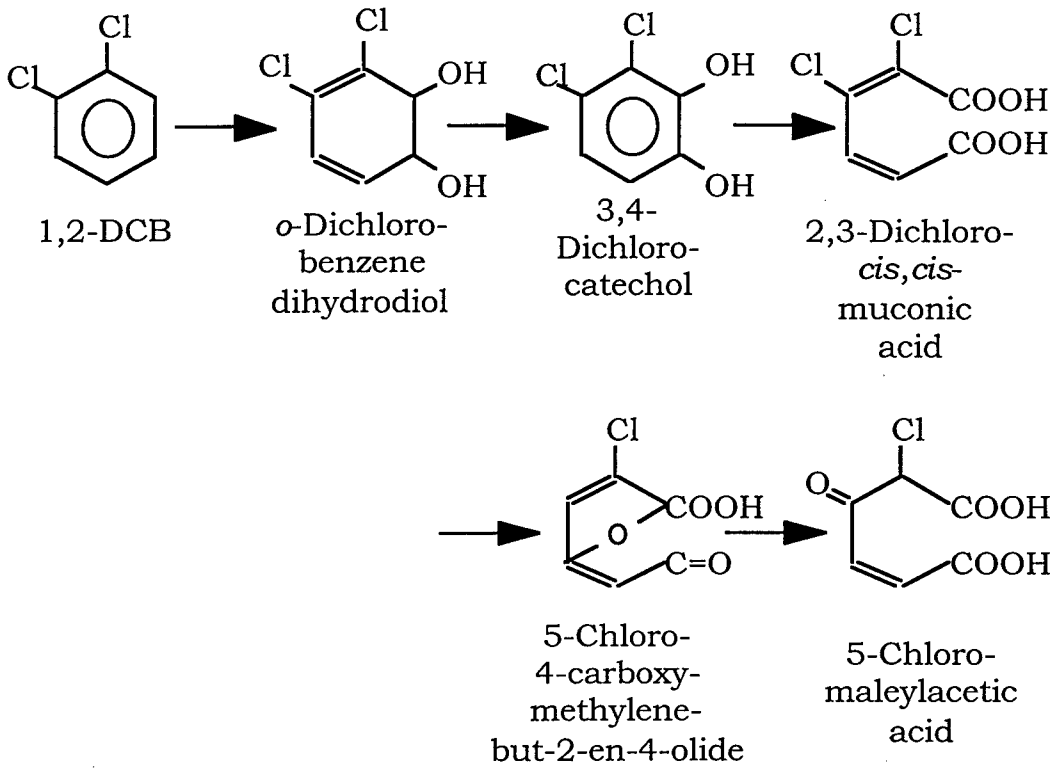


Figure 2.3 -- Proposed oxidation of 1,2-DCB by Haigler et al.

was isolated in pure culture and tentatively identified as a *Pseudomonas* species. The doubling time of the organism while growing on 1,2-DCB was 5.5 hours. The metabolic pathway was determined by a similar method to deBont et al. and is displayed in Figure 2.3 (Haiger et al., 1988).

Since these reports, there have been many studies on the oxidation of DCBs. All the known reports exhibit an oxidation pathway similar to those shown in Figures 2.1 through 2.3.

2.C Anaerobic Degradation of Chlorinated Benzenes

Bacterially mediated anaerobic degradation of poly-chlorinated benzenes has been found to occur via reductive dechlorination -- the

replacement of a chlorine atom with a hydrogen atom. Reductive dechlorination of chlorinated benzenes was first found in 1975 by Mehendale et al. They found slight amounts of pentachlorobenzene (PeCB) and tetrachlorobenzene (TeCB) after feeding a rat a single dose of HCB (Mehendale et al., 1975). A more complete study was conducted in 1984 by Tscuchiya et al. who isolated *Staphylococcus epidermidis* from the intestine of rats. This bacterium was able to reductively dechlorinate a slight amount of 1,2,4-trichlorobenzene (TCB) to MCB (Tschuchiya and Yamaha, 1984). Since then, there have been reports of chlorinated benzenes being reductively dechlorinated as far as DCB, MCB and one report of benzene formation from MCB.

2.C.1 Reductive Dechlorination of Chlorinated Benzenes to DCB

In 1987, Fathepure et al. observed reductive dechlorination of HCB in anaerobic sewage sludge. They added ca. 200 μM HCB to the fresh sludge and after a lag time of one week, they observed more than a 90% conversion to 1,3,5-TCB which then remained unchanged. They also observed small quantities of 1,2,4-TCB that were converted to primarily 1,2- and 1,3-DCB. They ran another study with 1,2,3,5-TeCB added to fresh sludge and found complete conversion to 1,3,5-TCB with no further change. In addition, they ran autoclaved samples and found no conversion of the chlorinated benzenes. From these experiments they hypothesized the

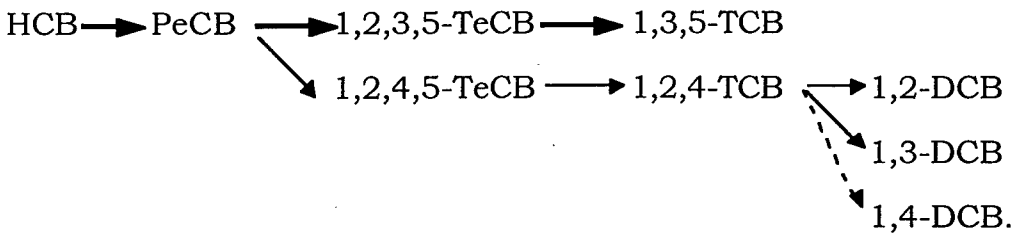


Figure 2.4 -- Proposed pathway for HCB reductive dechlorination by Fathepure et al. Bold arrows indicate the predominate pathway and dashed arrows indicate very minor pathways.

transformation pathway portrayed in Figure 2.4 (Fathepure et al., 1987).

In 1992, Holliger et al. found a mixed culture enriched from the Rhine river that dechlorinated all the TCB isomers to mostly 1,3-DCB. They found that H_2, lactate and ethanol worked best as electron donors. The temperature optimum for the culture was between 25 and 30°C. Addition of 2-bromoethanesulfonic acid (BESA), an inhibitor of methanogens, did not reduce the dechlorination rates. They also used the enrichments to test the dechlorination of TeCB, PeCB and HCB. They proposed the same pathway for HCB degradation as Fathepure et al (Figure 2.4). They conjectured that the reductive dechlorination of HCB down to DCB was carried out by several organisms because the source of the enrichment culture could dechlorinate all TCB and DCB isomers while the enrichment culture could only transform 1,2,3-TCB (Holliger et al., 1992).

In 1993, Beurskens et al. took sediment from the Rhine river and incubated it for 18 weeks with a high HCB addition (1.4 mmoles/kg soil). They found that 86% of the HCB was linearly consumed with significant increases in 1,3,5-TCB and 1,3-DCB. This

study was exciting because they used historic, frozen cores from 1972 and current cores from the same location and found a decrease in HCB and an increase in 1,3,5-TCB and 1,3-DCB. This study supports the idea that reductive dechlorination is occurring at sites (see Section 2.A for more details) (Beurskens et al., 1993).

Adrian et al. worked on optimizing a growth medium for a culture dechlorinating a mixture of 1,2,3- and 1,2,4-TCB to DCB (isomers not specified). The culture was obtained from the biomass covering the polyurethane foam body from a fluidized-bed reactor. Pyruvate was added as a carbon and energy source and after seven weeks dechlorination to DCB was observed.

When BESA was added to inhibit methanogens, they noted an increase in the rate of dechlorination. They also noted that when Ti(III) citrate was used as a reducing agent, instead of sulfide, dechlorination rates increased. In a seven-day study, enrichments in media using Ti(III) citrate dechlorinated 95.8% of the TCBs while only 3.7% of the TCBs were dechlorinated in media using sulfide. They also observed increased dechlorination when tungstate-selenite and cyanocobalamin were added and when the following six vitamins were excluded: p-aminobenzoate, biotin, nicotinic acid, pantothenic acid, pyridoxine and thiamine (Adrian et al., 1997).

In 1997, Susarla et al. mixed sediment and water from the Lake Kasumigaura and placed the slurry in test tubes. HCB was then added to the test tubes and they were incubated at 25°C. After a lag period of four days, HCB began to be transformed and was completely consumed after 225 days. 1,3- and 1,4-DCB were the

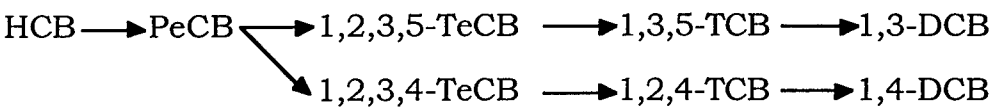


Figure 2.5 -- Proposed pathway for HCB reductive dechlorination by Susarla et al. The top pathway is under sulfidogenic conditions and the bottom pathway is under methanogenic conditions.

detected end products. Since the sulfate was completely consumed after 60 days, the authors postulated that during the first 60 days sulfidogenic bacteria were dominant, and after the sulfate was consumed methanogens became dominant. This transition in microbial populations led the authors to believe that HCB was transformed by two distinct pathways, possibly by two distinct groups of bacteria during the different conditions (Figure 2.5) (Susarla et al., 1997).

In 1998, Jackson and Pardue. studied the effects of metal inhibition on reductive dechlorination of HCB. They took samples from Devil's Swamp/Batton Rouge Bayou, a site known to be contaminated with HCB and metals. Dechlorination was observed for all live bottles except those fed the highest concentration of cadmium (Cd) (1000 mg/L). The average rate of HCB dechlorination in bottles not fed metals was 0.021/day with no lag period.

Cadmium and lead (Pb) were the most inhibitory metals followed by copper and zinc. The inhibitory effects of Cd on dechlorination appeared to be linear with respect to the log of added Cd and additions of 100 mg/kg caused an increase in the lag period to 30-40 days. Pb on the other hand seemed to be inhibitory over all tested additions, and an addition of 1000 mg/kg caused an increase in the lag period to 20-30 days.

They also studied the effects of cobalt and found that even though this metal might be an important component of a co-enzyme responsible for the dechlorination, it had no beneficial effect on dechlorination. Finally, they measured the effect of metal addition on methane production and found it to be less sensitive than dechlorination. The authors believed that the bacteria responsible for the reductive dechlorination may be more sensitive or more exposed to the metals than the methanogens (Jackson and Pardue, 1998).

In 1998, Chang et al. studied the effects of methanogenic, sulfate-reducing and denitrifying conditions on a culture capable of dechlorinating HCB to DCB. The culture was enriched from river sediment from sites contaminated with petrochemical effluent in Southern Taiwan. Originally the culture was adapted to 1,2,3-TCB, and after one month was able to dechlorinate HCB. One milliliter of this adapted culture was added to 9 ml of media in methanogenic, sulfate-reducing or denitrifying conditions. After two days, HCB was transformed in both the sulfate-reducing and methanogenic bottles. The dechlorination rates were 0.14 mg/L/day and 0.18 mg/L/day for sulfate-reducing and methanogenic conditions respectively. Chang et al. noted the same degradation pathway as Fathepure et al. (Figure 2.4). Dechlorination was never observed in the denitrifying bottles.

Much-reduced rates of dechlorination for both methanogenic and sulfate-reducing cultures were noted when high amounts (50 mg/L) of HCB were added. In addition, dechlorination

stopped when ferric chloride or manganese dioxide was added. Fe(III) and Mn(IV), may compete with chlorinated benzenes as electron acceptors. Enhanced dechlorination was observed when lactate or pyruvate was added as electron donors. However no effect was observed with acetate addition.

Dechlorination was hindered when BESA was added to the sulfate-reducing culture and completely stopped when both BESA and vancomycin (an inhibitor of Gram-positive bacteria) were added. Similarly, dechlorination was hindered when molybdate (an inhibitor of sulfate-reducers) was added to the methanogenic culture and completely stopped when both molybdate and vancomycin were added. These results suggest that several different populations were responsible for the observed dechlorination. Dechlorination rates were highest in methanogenic cultures (Chang et al., 1998).

2.C.2 Reductive Dechlorination of Chlorinated Benzenes to MCB

In 1988, Bosma et al. observed dechlorination of all TCB and DCB isomers to MCB. They packed PVC columns with sediment from the Rhine river and continuously ran a mineral media in an upflow manner through the columns. Na_2S was added to the medium to maintain reduced conditions. Initially the TCB and DCB isomers were continuously fed to the column. After 2 - 6 months the TCB isomers were no longer detected in the effluent. 1,2,3-TCB was removed within the first 5 cm of the 25 cm column, 1,2,4-TCB was removed within the first 10 cm and 1,3,5-TCB was removed within

the first 20 cm. Similar experiments were also conducted with the individual TCB isomers and they were able to determine that 1,3-DCB was formed from 1,2,3- and 1,3,5-TCB while 1,4-TCB was formed from 1,2,4-TCB.

After 450 days of operation, DCBs were continuously fed to the column instead of TCBs. After seven days the DCBs were dechlorinated to MCB. The maximum dechlorination rate obtained for DCB dechlorination was 0.4 μM/hour. Only after the complete transformation of 1,2-DCB to MCB could 1,3- and 1,4-DCB begin to be dechlorinated. In addition none of the DCB isomers could be dechlorinated when TCBs were present (Bosma et al., 1988).

In 1993, Ramanand et al. added a mixture of HCB, PeCB and 1,2,3-TCB to mineral media and soil from near Niagara Falls, New York. They noted an almost complete transformation of the chlorinated benzenes to MCB with no noticeable accumulation of any of the TCB or DCB isomers. Figure 2.6 shows their proposed pathway (Ramanand et al., 1993).

In 1996, Masunaga et al. studied the dechlorination of chlorobenzenes from HCB down to MCB in separate experiments using groundwater and sediment from the mouth of the Tsurumi river. The byproduct formation was measured in each test tube for a

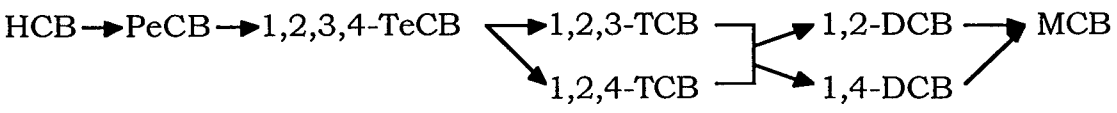


Figure 2.6 -- Proposed pathway for HCB degradation by Ramanand et al.

HCB ⟶ PeCB ⟶ 1,2,4,5-TeCB ⟶ 1,2,4-TCB ⟶ 1,4-DCB ⟶ MCB.

Figure 2.7 -- Proposed pathway for HCB dechlorination by Masunaga et al.

period of about 325 days and MCB was observed in each case. From this data they proposed the major pathway for HCB transformation expressed in Figure 2.7 (Masunaga et al., 1996).

In 1997, Middeldorp et al. incubated a mixture of sediment from the Rhine river, dredging sludges polluted with oil, sediment from Lake Ketelmeer and granular sludge from a wastewater treatment plant in the presence of PeCB and found chloride production after 40 days. The culture was then transferred several times and tested individually with each of the chlorinated benzenes except MCB. From these tests they proposed a pathway for PeCB degradation (Figure 2.8). In the freshly transferred cultures they found that there was a 12-day lag period for PeCB dechlorination but less than a two day lag period for the other, less-chlorinated compounds tested.

In addition they tested various electron donors and found that lactate, glucose and propionate resulted in the highest rate of dechlorination. Ethanol, methanol, hydrogen and acetate also supported dechlorination, while formate did not.

When BESA was added to the media, methanogenesis was suppressed while dechlorination towards MCB continued. Under these conditions lactate was still converted to propionate and acetate. They proposed that BESA inhibited some of the dechlorination branches because the microbes responsible were

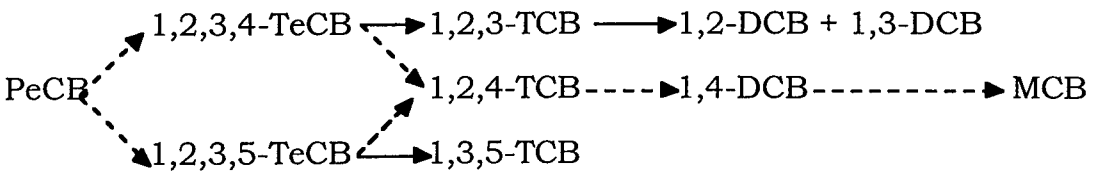


Figure 2.8 -- Proposed pathway for PeCB degradation by Middeldorp et al. Dashed arrows indicate reactions that proceed in the presence of BESA

methanogens or were dependent on methanogens (Figure 2.8). An additional explanation could be that BESA actually hindered the dechlorination. These results suggested that more than one microbial population was responsible for the observed transformations of PeCB to MCB or that multiple pathways existed within a single organism.

In a four-day experiment, they tested the temperature optimum for dechlorination of 1,2,4-TCB and found no dechlorination at 4, 10 and 55°C, equal dechlorination at 20 and 30°C and about half maximum dechlorination at 37°C. These results implied in situ degradation was probably hindered by the temperature of 10°C at the site (Middeldorp et al., 1997).

2.C.3 Reductive Dechlorination of Chlorinated Benzenes to Benzene

So far, there is one known report of chlorinated benzenes reductively dechlorinated to benzene. Sediment from the Saale river in Germany was used as the inoculum for Nowak et al.'s chlorobenzene degradation experiment. Due to continual methane and CO_2 production, they surmised that their mixed culture was

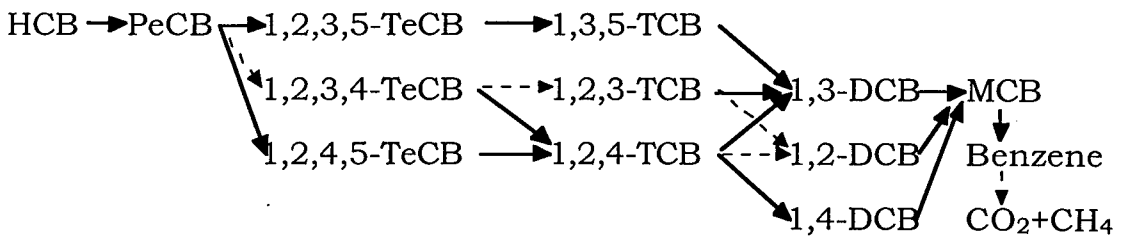


Figure 2.9 -- Proposed pathway for HCB reductive dechlorination by Nowak et al. Dashed arrows indicate minor pathways and the final step from benzene to CO_2 and CH_4 is not certain.

methanogenic. The serum bottles were fed 0.5 mmol/L of the individual chlorinated benzenes which were all degraded to MCB (Figure 2.9). From these experiments they observed that the removal of a chlorine in the ortho position to another chlorine was favored.

After about 24 days, they observed a slight increase in benzene concentrations (while using a FID) and confirmed its presence using a GC/MS. They found accumulations of 0.05 mmol/L of benzene and only noticed benzene accumulation while the culture was degrading a chlorinated benzene with at least two chlorines. They postulated that the benzene formation was due to the microbes preferably using the more chlorinated benzenes and transforming MCB as a co-substrate. They also proposed that the organisms that transform more-chlorinated benzenes could provide the MCB degraders with vital intermediary products.

When Nowak et al. inhibited methanogenesis with BESA they found that dechlorination continued but at a much slower rate. Thus methanogens were an advantageous part of the system or BESA inhibited dechlorination itself.

In addition, they tested electron donors and found that pyruvate increased the rate of dechlorination by 300% compared to those cultures fed no electron donor. Methanol, ethanol, acetone and acetate all increased the rate of dechlorination but to a lesser degree and are presented in order of electron donor ability (Nowak et al., 1996).

CHAPTER THREE -- METHODS

3.A Experimental Strategy

This project had two main goals. Since a majority of the funding came from the US Air Force as part of a natural-attenuation study, the first goal was determine whether DCBs could be anaerobically degraded by native bacteria at the contaminated sites. The second goal was to find and enrich for a DCB dechlorinating microbe.

This experiment was conducted using samples from eight different locations: Plattsburgh Air Force Base (P), Kelly Air Force Base (K), Robins Air Force Base at well BIA4 at 17 feet (R-1-s) and at 25 feet (R-1-d), Robins Air Force Base at well R13 (R-2), Robins Air Force Base landfill leachate (R-LF), digested sludge from the Ithaca wastewater treatment plant anaerobic digester (DS) and wetland sediment from a contaminated site in Louisiana (L). The first four sites, P through R-1-d, were part of the Air Force natural-attenuation study.

Microcosms were prepared in an anaerobic glovebox by adding soil and groundwater from each site to multiple serum bottles. Since the study consisted of two goals, several different types of microcosms were constructed for each site. All microcosms received all three DCB isomers, but ambient levels of each DCB were added to the natural-attenuation study sites (~ 1 μmole/bottle) and high levels of each DCB were added to all other sites to aid in dechlorinator growth (~ 20 μmole/bottle). Two different electron

donor conditions were studied. Some microcosms were prepared with added electron donor in the form of yeast extract (YE), and some without added electron donor. The microcosms without electron donor were constructed to imitate in situ conditions (and thus the goals of the natural-attenuation study), while those with added electron donor were constructed to aid in the stimulation of dechlorination. Microcosms were also prepared with autoclaved soil ("autoclaved soil controls") to distinguish biotic versus abiotic transformations. Finally, autoclaved water controls accompanied the other microcosms to determine the role of sorption (through comparison with autoclaved soil controls).

The microcosms were then monitored for the DCB isomers, MCB, benzene, CH_4 and H_2 using headspace injections onto a gas chromatograph (GC) system equipped with a flame-ionization detector (FID), thermal-conductivity detector (TCD) and a reduction-gas detector (RGD). Bacterially mediated reductive dechlorination of DCBs was determined by noting a decrease in DCB concentration while noting a corresponding increase in MCB concentration in live microcosms, while observing no change in DCB concentrations in autoclaved controls.

Once reductive dechlorination was observed, some of the soil microcosms were enriched for the responsible bacteria by adding more DCBs. After sufficient data had been gathered for those microcosms that were part of the natural-attenuation study, high levels of DCBs were added to those too.

The electron donor available in the microcosms was monitored by injecting liquid samples to a GC system equipped with an FID to measure volatile fatty acids (VFAs). Once the electron donor was depleted more YE was added.

After a few DCB feedings, a small inoculum was taken from these microcosms and added to a serum bottle filled with an anaerobic basal medium. The basal-medium enrichments were monitored identically to the soil microcosms. The goal of these basal-medium enrichments was to develop a well-defined system in which the bacteria responsible for the DCB dechlorination could be enriched for and studied.

3.B Source Material

Since the microbes responsible for the hypothesized reductive dechlorination would be expected to be anaerobes, strict anaerobic conditions were maintained while collecting the source material. Before taking a soil sample, a standard canning jar was first filled with groundwater from the site. Then the soil was added to the canning jar, displacing the groundwater. This continued until the jar was filled almost to the top. Then the jar was topped off with groundwater, the sides of the jar, threads and lip were wiped clean, and the jar was sealed with its lid and retainer ring.

3.B.1 Plattsburgh Air Force Base

Soil and groundwater from Plattsburgh Air Force Base, in New York, were collected on May 2, 1996 by Dr. James Gossett. The soil

and groundwater were collected near well 84DD at a depth of 25 feet by a geoprobe. Once the geoprobe had been punched into the ground to the desired depth, a polyethylene tube was snaked into the piping. Then the soil and groundwater slurry was pumped out of the ground and passed trough a flask to separate out the soil from the groundwater. Well 84DD was at the bottom of a drainage ditch and had 11.13 nM of H_2 present. There was evidence of reductive dechlorination of trichloroethene (TCE), since all the TCE was gone from the site but *cis*-dichloroethene and vinyl chloride were present. Plattsburgh samples are designated P in this thesis.

3.B.2 Kelly Air Force Base

Soil and groundwater were collected from Kelly Air Force Base, in San Antonio, on March 24, 1998 by Dr. James Gossett with assistance from Todd Herrington of Parson Engineering Systems. The samples were collected from site S-1 from 27 to 28 feet below the ground surface at a location on the B-B' cross section at or near SB199. Groundwater was obtained from nearby SS003W050. The samples are designated K in this thesis.

3.B.3 Digested Sludge from the Ithaca Wastewater Treatment Plant

Anne Quistorff collected anaerobic digester sludge (DS) from the Ithaca Area Wastewater Treatment Plant on June 28, 1998.

3.B.4 Louisiana Sediment

Dr. Spyros Pavlostathis, from Georgia Tech, collected surface sediment from a wetland area in Louisiana known to be impacted by surrounding petrochemical and agrochemical industries. The material had been collected several years earlier at the intersection of the Calcasieu river in southwestern Louisiana. Louisiana sediment samples are designated L in this thesis.

3.B.5 Robins Air Force Base Landfill Leachate

Anne Quistorff collected the Landfill-3 leachate on October 7, 1998 by bailing from the Landfill-3 extraction well. The samples are designated R-LF in this thesis.

3.B.6 Robins Air Force Base at Well R13

Anne Quistorff collected soil and groundwater from near well R-13 on October 7, 1998. Soil was collected about one foot away from well R13-2W by hand auguring to below the groundwater table. Groundwater was collected from well R13-2W by bailing. Two well volumes were removed first before the samples were collected. The samples are designated R-2 in this thesis.

3.B.7 Robins Air Force Base at Well BIA4 at 17 and 25 Feet

Soil and groundwater were collected by RUST employees on October 27, 1998. Soil samples were collected in the process of drilling a new well (BIA4), and groundwater samples were collected from a nearby well R15-7W. The soil samples were collected by the

process mentioned in Section 3.B, except that the some of the samples were collected in small glass jars with Teflon-lined caps. The shallow and deep samples from this location are designated R-1-s and R-1-d, respectively, in this thesis.

Table 3.1 contains a summary of the sites and their abbreviations.

3.C Preparation of Microcosms

Microcosms were prepared inside an anaerobic glovebox (~2% H_2, 98% N_2 atm) for all sites except the DS samples. First, moisture-content was determined for subsurface soil material (see 3.G.3). For P microcosms the goal was to have 50 g of soil (on a dry weight basis) and 50 g of water. From the moisture-content measurement, sufficient wet weight of soil was added to yield 50 g of dry weight. Then sufficient groundwater was added such that, taking into account soil moisture and DCB stock additions (4 g) (3.G.1a), 50 g of water was present. The same process was used for R-1-s and R-1-d microcosms except that the goal was to have 100 g of water and the DCB stock addition was 8 g. The same process was also used for K, and R-2 microcosms, except that the goal was 100 g of water and that there was no DCB stock addition. Since there was no soil for the R-LF microcosms, 100 g of the leachate was added to the serum bottles inside the glovebox. The groundwater used in all microcosms was laced with 1 mg/L resazurin, a redox indicator. For L microcosms, all the available soil slurry was added in equal amounts

Table 3.1 -- Site Summary

	Abbreviation	Site Classification	Comments
Plattsburgh Air Force Base	P	natural attenuation site	
Kelly Air Force Base	K	natural attenuation site	LNAPL present, large rocks
Digested Sludge	DS	not a natural attenuation site	
Louisiana Sediment	L	not a natural attenuation site	
Robins Air Force Base Landfill Leachate	R-LF	not a natural attenuation site	not very reduced
Robins Air Force Base Well R13-2W	R-2	not a natural attenuation site	low pH, low alkalinity
Robins Air Force Base Well BIA4 at 17 feet	R-1-s	natural attenuation site	
Robins Air Force Base Well BIA4 at 25 feet	R-1-d	natural attenuation site	

to each microcosm and then basal media was added to each microcosm to attain a total mass of 106 g.

Once the groundwater was added, the microcosms were sealed with autoclaved Teflon-backed, gray-butyl rubber septa (Wheaton Industries) and crimped with aluminum crimp caps. The microcosms were then taken out of the glovebox.

The DS bottles were created by adding 92 g of sludge to 5 x 160-ml serum bottles. The bottles were capped and crimped as described above.

Twelve microcosms were prepared for each site (except DS). The first four were fed YE (to a concentration of 100 mg/L) and DCBs. YE was added by a sterile, plastic, 1-ml syringe from the YE stock (3.G.1c). Since the volume of stock added (0.2 ml) did not significantly contribute to the total volume of water, this volume was not included in the total water tally. Before January 26, 1999, whenever a microcosm was being fed both DCBs and YE, they were added on the same day. After January 26, 1999, the YE was added one day after the DCBs had been added, during which time the microcosm was equilibrating on a wrist-action shaker. Microcosms #5 through #7 were given only DCBs. Microcosms #8 and #9 were fed DCBs and autoclaved for 45 minutes, three times, 24 hours apart. These bottles served as autoclaved soil controls. Microcosms #10 though #12 were water controls and consisted of 100 ml water (autoclaved for 45 minutes) to which neat DCBs or the DCB stock was later added. For the DS microcosms, all five bottles were given

DCBs and none were given YE due to the high organic content of the sludge. In addition, none of these microcosms were autoclaved.

Since P, R-1-s and R-1-d microcosms were part of a natural-attenuation study, they were given low concentrations of DCBs to model in situ conditions. Low levels of DCBs were also employed with DS microcosms. DCBs were at first added to these microcosms from the DCB stock (see 3.G.1a), resulting in a total (nominal) DCB concentration of 4.2 mg/L (3 µmol/bottle). The microcosms were agitated for 24 hours on a wrist-action shaker.

Microcosms from the other sites received high levels of DCBs in neat form. (K microcosms were also part of a natural-attenuation study, but since the site contained a separate light non-aqueous-phase liquid (LNAPL), high levels of DCBs were required for analytical convenience.) Some microcosms had high background DCB levels. These were taken into account; enough neat DCBs were added to reach a final level of 20 µmole/bottle of each DCB isomer. The resulting nominal concentration of total DCB was thus 100 mg/L (60 µmol/bottle). These microcosms were agitated on a wrist-action shaker for one week. All microcosms were then stored quiescently, inverted at 24°C in the dark. Table 3.2 contains the volumes and masses of DCBs added for each site.

Over-pressure was added to each microcosm when deemed necessary. This was done by flushing a sterile 10-ml BD Glaspak syringe with N_2 or N_2/CO_2 several times to remove any O_2 from the syringe. Then 5 ml of the gas was added to each microcosm after first sterilizing the septum (3.F). The septa were also changed when

Table 3.2 -- Microcosm additions for each site

Site	dry soil (g)	ground water (g)	DCB stock (ml)	neat 1,4-DCB (mg)	neat 1,2-DCB (µl)	neat 1,3-DCB (µl)
P	50	46	4	--	--	--
DS	2.31	89.69	8	--	--	--
R-1-s	50	92	8	--	--	--
R-1-d	50	92	8	--	--	--
K	50	104	--	3	2.3	2.3
L	1.96	76.18*	--	3	2.3	2.3
R-LF	0	100	--	2.3	--	1.6
R-2	50	100	--	3	2.0	2.3

* L also received a 30.08 g addition of basal media.

deemed necessary. This was done by removing the old aluminum crimp cap and septum while at the same time inserting a sterile canula into the headspace though which either N_2 or N_2/CO_2 was flowing. Then a new autoclaved and sterilized septum was placed on the serum bottle while the canula was removed. The microcosm was sealed with an aluminum crimp cap.

Due to the low alkalinity present in R-1-s and R-1-d and the repetitive additions of YE that several microcosms received, alkalinity in the form of $NaHCO_3$ was directly added to some microcosms. This was done by weighing a 10-ml glass vial and crimping assembly and then adding 0.336 g of $NaHCO_3$. The vial was then crimped and autoclaved. The crimp cap and septa were removed from the microcosm while a sterile canula was inserted into the headspace through which N_2/CO_2 was flowing. The seal on the vial with the $NaCO_3$ was then removed and the $NaCO_3$ was added to the microcosm with a sterile spatula. The microcosm was then sealed.

3.D Enrichment Cultures

Once reductive dechlorination was evident in R-1-s, R-1-d, L and K microcosms, enrichment cultures were prepared from them. Enrichments from R-1-s and R-1-d microcosms were made by adding 84 ml of basal medium (3.G.1f) to 160-ml serum bottles while purging the bottles with anaerobic-grade N_2 (3.G.1e). The bottles were then capped with autoclaved Teflon-backed gray-butyl-rubber septa and crimped. The bottles were then autoclaved for 45 minutes. Then 8 ml of the silty-liquid from R-1-s and R-1-d microcosms were transferred, using sterile syringes, to the basal-medium serum bottles. The basal-medium bottles were then fed 8 ml of DCB stock (achieving a total DCB level of ~3 μmole/bottle) and 100 mg/L of YE from its stock. Once the first dose of DCBs had been consumed and MCB had formed, neat DCBs were added to each enrichment.

Enrichments from L were prepared in three 160-ml serum bottles containing 99, 98 and 95 ml of basal medium, respectively. They were autoclaved, then transported into the glovebox, along with an active microcosm that served as source culture for preparation of the enrichments. The septa were removed from all of the bottles and 1, 2 and 5 ml of mixed content from the L microcosm were added to the 99, 98, and 95 ml basal-medium bottles. Eight milliliters of autoclaved basal medium was added back to the L source microcosm. These bottles were then capped and removed from the glovebox. Neat DCBs and YE from the stock were added to each of these enrichments.

Enrichments from K microcosms were made by a similar method except the source material was transferred into the basal medium on the bench top while under a N_2 purge. These microcosms were constructed on the bench top due to the concern that the source microcosms would not be able to withstand the vacuum to which they would be subjected in the airlock leading to the glovebox. This was a concern for K microcosms because the source material contained many rocks and during the week of agitation of the wrist-action shaker hair-line fractures developed on the bottles. These fractures caused three microcosms to break in the beginning of the experiment.

The basal-medium enrichment microcosms were stored quiescently, at 24°C in the dark.

3.E Anaerobic Methods

Once the soil and groundwater were transported to the lab, they were stored at 5°C. The soil and groundwater canning jars were only opened in the anaerobic glovebox. The microcosms were also prepared and sealed inside the glovebox. Anytime the seal of the microcosm had to be removed outside the glovebox, anaerobic conditions were maintained by purging the headspace with sterile N_2 using a canula.

The water used in the DCB stock, YE stock and resazurin stock was purged with N_2 to remove any O_2. In addition, before any liquid was removed from an anaerobic stock or microcosm, an equal

amount of N_2 was added to the stock or microcosm so that air would not be drawn into the bottle.

3.F Sterile Methods

Since microcosms existed from many different sites, it was important to maintain sterility so as not to contaminate one site with organisms from another. Sterility was maintained by autoclaving any instrument that touched the soil (such as spoons and spatulas) or entered the microcosms (such as needles from syringes). All syringes used to add over-pressure, add stocks or transfer cultures were autoclaved before each use. In addition, before any needle punctured a septum, the top was doused in ethanol and ignited to kill any microbe that might be living on the septum. When withdrawing headspace samples from the microcosms for GC analysis, only the needle was sterile; therefore samples were withdrawn without ever expelling gas back into a microcosm.

The purge gas was sterilized by placing cotton plugs in the tubing before the canulas and the diffusing stones. In addition the tubing, canulas and diffusing stones were autoclaved before each use.

3.G Analytical Methods

3.G.1 Reagents and Solutions

1,2-DCB (Aldrich Chemical Co., 99% HPLC grade), 1,3-DCB (Aldrich Chemical Co., 98%) and 1,4-DCB (Aldrich Chemical Co.,

99+%) were initially directly added to R-2, R-LF, K, and L microcosms. Benzene (Fisher Scientific Co., certified A.C.S.) and MCB (Fisher Scientific Co., certified), the previously mentioned DCBs, CH_4 (Scott Specialty Gases), H_2 (Matheson Gas Products, 1% in N_2 and ultra high purity grade) were used in the preparation of analytical standards. Sulfuric Acid (LabChem Inc., 5.0 N) was used to make a 0.1 N solution for alkalinity titrations. Phosphoric Acid (Fisher Scientific Co., 85% Certified A.C.S.) was used to make a 1 N and 8 N solution for acidifying liquid samples prior to GC injection for VFA analysis. Ethanol (campus supplier, 95% by volume) was used to sterilize septa before needle puncture.

3.G.1a DCB Stock Solution Since the DCBs have a very low solubility in water (0.147 g/L for 1,2-DCB, 0.106 g/L for 1,3-DCB and 0.0829 g/L for 1,4-DCB (Lide, 1998)), it would be impractical to add the small volumes required for the low-concentration microcosms. For the low-concentration, DCB-amended microcosm (P, R-1-s, R-1-d and DS) a stock solution was prepared. The stock was made by filling a 1-L serum bottle with 800 ml of distilled water and a Teflon stir bar, then purging with N_2 for one hour to remove any O_2. Then 15.5 mg of 1,4-DCB and 12 µl each of 1,2-DCB and 1,3-DCB were added. The stock jar was sealed with a previously autoclaved Teflon-backed, gray-butyl rubber septa and crimped with an aluminum crimp cap. The solution was then autoclaved for one hour and then mixed on a stir plate overnight. The resulting liquid concentrations for the stock used for P microcosms were 19.5 mg/L for 1,2-DCB, 18.8 mg/L for 1,3-DCB and 18.5 mg/L for 1,4-DCB. The liquid concentrations

for the stock used for R-1-s, R-1-d and DS microcosms were 19.3 mg/L for 1,2-DCB 19.2 mg/L for 1,3-DCB and 18.2 mg/L for 1,4-DCB. These concentrations were determined by first adding known masses of the DCB methanol stock (3.G.1b) to 100 ml of water in a 160-ml serum bottle and performing a calibration on the GC (3.G.4a). Once the calibration was completed 8 ml of the DCB stock solution was added to 92 ml of water in a 160-ml serum bottle. The concentration of DCBs was then determined from the previous calibration.

3.G.1b DCB, MCB and Benzene Stocks in Methanol

Methanol DCB, MCB and benzene stocks were used to standardize the GC system. Amounts added to the stocks were gravimetrically determined. The DCB methanol stock was made by weighing a 20-ml serum vial and cap assembly empty and after each addition. First, 10 ml of methanol (Fisher Scientific Co., HPLC grade) was added to the 20-ml serum vial. Then a known mass of 1,4-DCB was added. The vial was then capped with Teflon-backed, gray-butyl rubber septa, crimped with an aluminum crimp cap. Then both 1,2-DCB and 1,3-DCB were added. Known amounts of this stock (less than 100 µl) were then added to 100 ml of water in a 160-ml serum bottle or to soil microcosm standards to calibrate the GC. The MCB and benzene methanol stocks were made by weighing a 20-ml serum vial and cap assembly empty and after each addition. First 10 ml of methanol was added to the vial, capped with a Teflon-backed, gray-butyl rubber septa, and crimped with an aluminum crimp cap. Then MCB and benzene were added.

3.G.1c Yeast Extract Solution The yeast extract solution was created by purging 100 ml of distilled water in a 160-ml serum bottle with N_2 to remove any O_2 and adding 5 g of powered yeast extract (Difco Laboratories). The serum bottle was then capped with a previously autoclaved Teflon-backed, gray-butyl rubber septum, crimped with an aluminum crimp cap and autoclaved.

3.G.1d Resazurin Solution The resazurin solution was made by purging 50 ml of distilled water in a 160-ml serum bottle with N_2 to remove any O_2 and adding 82 mg resazurin (Difco Laboratories). The serum bottle was then capped with a previously autoclaved Teflon-backed, gray-butyl rubber septum, crimped with an aluminum crimp cap and autoclaved.

3.G.1e Titanium Chloride Scrubbing Solution Anoxic gas (either N_2 or a mixture of N_2 and CO_2) used to purge microcosms and solutions was continuously bubbled through a titanium chloride scrubbing solution to remove any O_2. The scrubbing solution was made by dissolving 10 ml of 20% titanous chloride solution (Fisher Scientific Co.), 12.5 g sodium bicarbonate (Fisher Scientific Co.), and 4.412 g of citric acid trisodium salt dihydrate (99%, Aldrich Chemical Co., Inc.) in 1 L of distilled water (Zehnder and Wuhrmann, 1976).

3.G.1f Basal Salts Medium Enrichment cultures from the R-1-s, R-1-d, L and K microcosms were grown in a basal salts medium described by Fennell (Fennell 1998). Please see Table 3.3 for the composition of the medium.

Table 3.3 -- Basal Salts Medium Composition

Compound	Quantity (per L distilled water)
NH_4Cl	0.2 g
$K_2HPO_4 \cdot 3H_2O$	0.1 g
KH_2PO_4	0.055 g
$MgCl_2 \cdot 6H_2O$	0.2 g
Resazurin	0.001 g
Trace Metals Solution*	10 ml
$FeCl_2 \cdot 4H_2O$	0.1 g
$Na_2S \cdot 9H_2O$	0.5 g
$NaHCO_3$	6.0 g

* Trace metal solution consisted of 0.1 g/L $MnCl_2 \cdot 4H_2O$, 0.17 g/L $CoCl_2 \cdot 6H_2O$, 0.1 g/L $ZnCl_2$, 0.251 g/L $CaCl_2 \cdot H_2O$, 0.019 g/L H_3BO_3, 0.05 g/L $NiCl_2 \cdot 6H_2O$, 0.02 g/L $Na_2MoO_4 \cdot 2H_2O$ and was adjusted to pH 7 with 8 N NaOH.

3.G.2 Syringes

Due to the extreme sorptive properties of MCB and DCBs to Teflon, the syringe used for GC analysis was changed on July 28, 1998 from a 0.25-ml VICI Pressure-Lok syringe to a 1-ml B-D Glaspak syringe. On August 11, 1998 the syringe was again changed to a 1-ml plastic B-D syringe with a Teflon Mininert valve.

A 10-ml B-D Glaspak syringe was used to deliver the DCB stock to the microcosms, and to add over-pressure to the microcosms. A 50-ml B-D Plastipak syringe was used to deliver over-pressure to the DCB stock solution. Hamilton Microliter syringes were used to deliver neat 1,2-DCB and 1,3-DCB to the DCB stock and to microcosms. A plastic B-D 1-ml syringe was used to take liquid samples from the microcosms for VFA analysis. VICI Pressure-Lok syringes were used to deliver neat MCB and DCBs to

the methanol stock and CH_4 and H_2 to 100 ml of water for GC standards.

3.G.3 Moisture Content Analysis

The moisture content of the soil from each site was determined following Standard Methods (American Public Health Association et al., 1975). First, three crucibles were ignited in the muffle furnace for one hour then placed in a desiccator overnight. Then about 60 g of soil was added to a jar inside the glovebox. Once the jar was removed from the glovebox, the three crucibles were weighed and about 20 g of soil was added to each crucible and they were weighed again. The crucibles were then placed in a 105°C oven overnight. The next day the crucibles were removed from the oven and placed in a desiccator for one hour and then weighed again. To insure that all the water had evaporated, the crucibles were placed in the oven for another hour, then in the desiccator for an hour and weighed again. Please see equation 1 for the equation used to determine the percent moisture in the soil.

$$\text{percent moisture} = \frac{\text{mass wet} - \text{mass dry}}{\text{mass wet} - \text{mass crucible}} \times 100 \qquad (1)$$

Where mass wet equals the mass of the crucible and soil before drying, mass dry equals the mass of the crucible and soil after drying, and mass crucible equals the mass of the crucible alone.

3.G.4 DCB, MCB, CH_4, H_2 Analysis

Headspace samples (0.5 ml) were analyzed for DCBs, MCB, benzene, CH_4 and H_2 on a system consisting of two GCs, an

additional stand-alone detector and a PC used for data acquisition. The two GCs and the stand-alone detector were linked through transfer lines. Both GCs were Autosystem GCs made by Perkin Elmer. The first GC (GC 1) had an electron-capture detector (ECD) and a flame-photometric detector (FPD) which was not used. The ECD could be used to measure DCBs and MCB. The second GC (GC 2) had a flame-ionization detector (FID) and a thermal-conductivity detector (TCD). The FID was used to measure CH_4, benzene, MCB and all three DCB isomers. The TCD was used to measure high levels of H_2. The stand-alone detector was a reduction-gas detector (RGD) made by Trace Analytical and was used to measure low levels of H_2.

DCBs and MCB were separated on a Supelco SP1000 80/100 Supelcoport 20-feet X 1/8-inch stainless-steel column. CH_4 and H_2 were separated on a 80-inch X 1/8-inch stainless-steel 60/80 Molecular Sieve by Supelco. The SP1000 column was inside GC 1 and the Molecular Sieve (MS) was inside GC 2.

The carrier gas for all detectors was N_2. The N_2 stream first passed through a charcoal column (Alltech Associates Inc.) and then passed though a Zeolite - Drierite column (Alltech Associates Inc.) to both purify and dry the gas. Since the RGD detects trace H_2, the N_2 gas stream then passed through a catalytic combustion filter (Trace Analytical) that removed H_2. This process produced water, so the N_2 stream then passed through a Carrier Gas Drying Tube (Supelco). Since the ECD was extremely sensitive to O_2, the carrier gas then passed though an Oxy-Trap (Alltech Associates Inc.) and

then an Indicating Oxy-Trap (Alltech Associates Inc.) both of which removed O_2 from the carrier gas. The carrier flow was set to 30 ml/min.

Air and H_2 were used to maintain the FID flame. Both these gas streams passed through the charcoal column and the Zeolite - Drierite column to dry and purify the gases. The air flow was set to 450 ml/min and the H_2 flow was set to 45 ml/min.

Between the two GCs, three 10-port valves (of which only four ports were used) guided the sample injected into GC 1 to the two columns and the three detectors (Figure 3.1). H_2 was the first chemical to be eluted and passed through the SP1000 and the MS to be analyzed on both the TCD and the RGD. This was accomplished by routing the effluent carrier gas stream from the SP1000 to valve one (V1), to the MS, to valve three (V3), to the TCD and finally to the RGD. The carrier flow continued in this manner until CH_4 entered the MS at 2.45 minutes. At this time, V1 switched and the flow from the SP1000 was routed to valve two (V2) and finally to analysis on the ECD. During this time, small chlorinated molecules such as vinyl chloride were analyzed on the ECD. Once H_2 had been analyzed on the RGD, at 3.6 minutes, V3 switched and the MS effluent was routed to V3, to V2 and then to the FID so that CH_4 could be analyzed on the FID. At 5 minutes, once CH_4 had been analyzed, V2 and V3 switched. This connected the SP1000 with V1, then V2 and then the FID. During this final stage, benzene, MCB, and the DCB isomers could be measured on the FID (Figure 3.2).

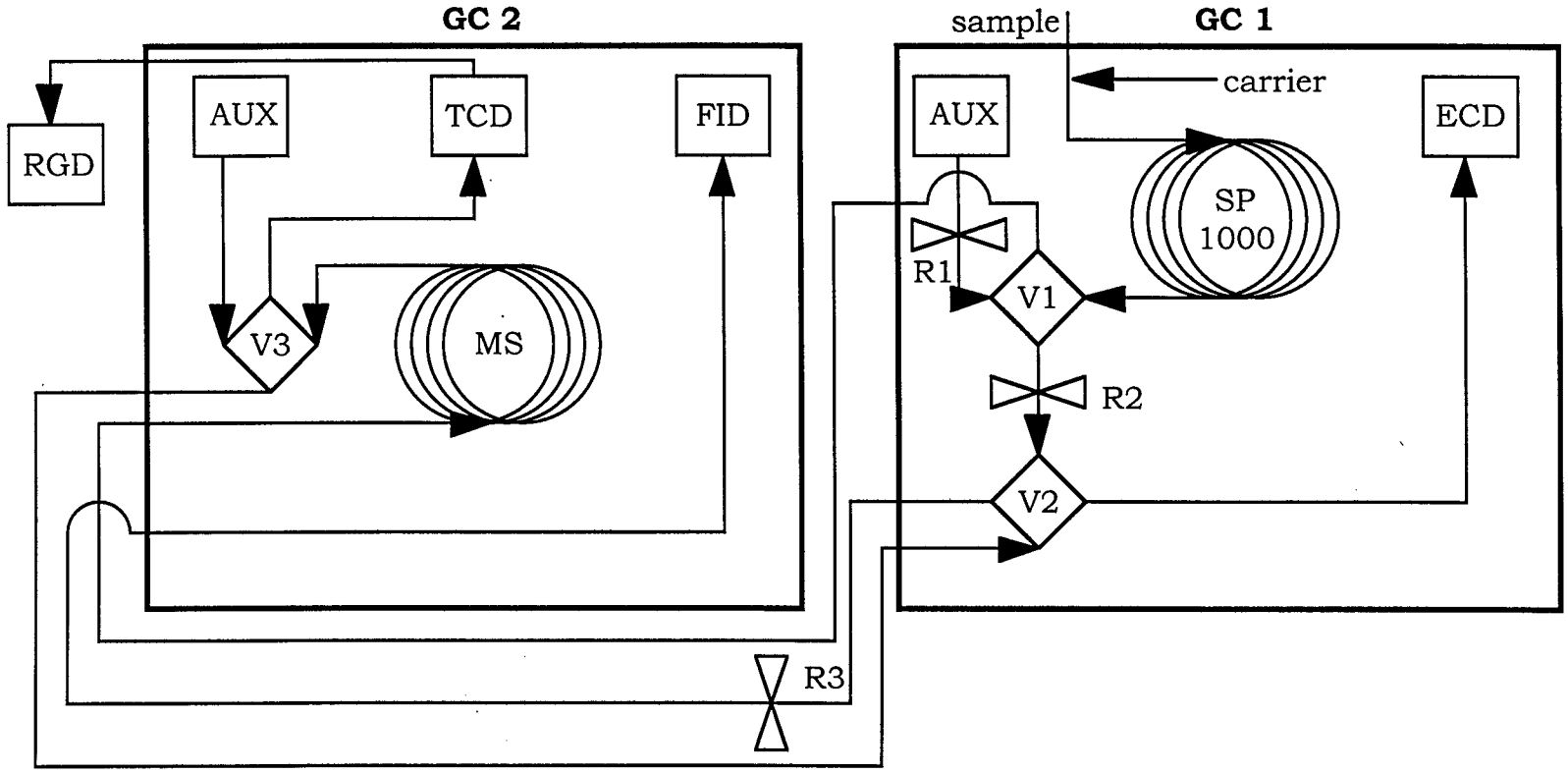


Figure 3.1 Schematic Diagram of the GC system for measuring DCBs. Where V1 - V3 represent valves 1-3, R1 - R3 represent restrictors 1-3 and AUX represent the auxiliary flows.

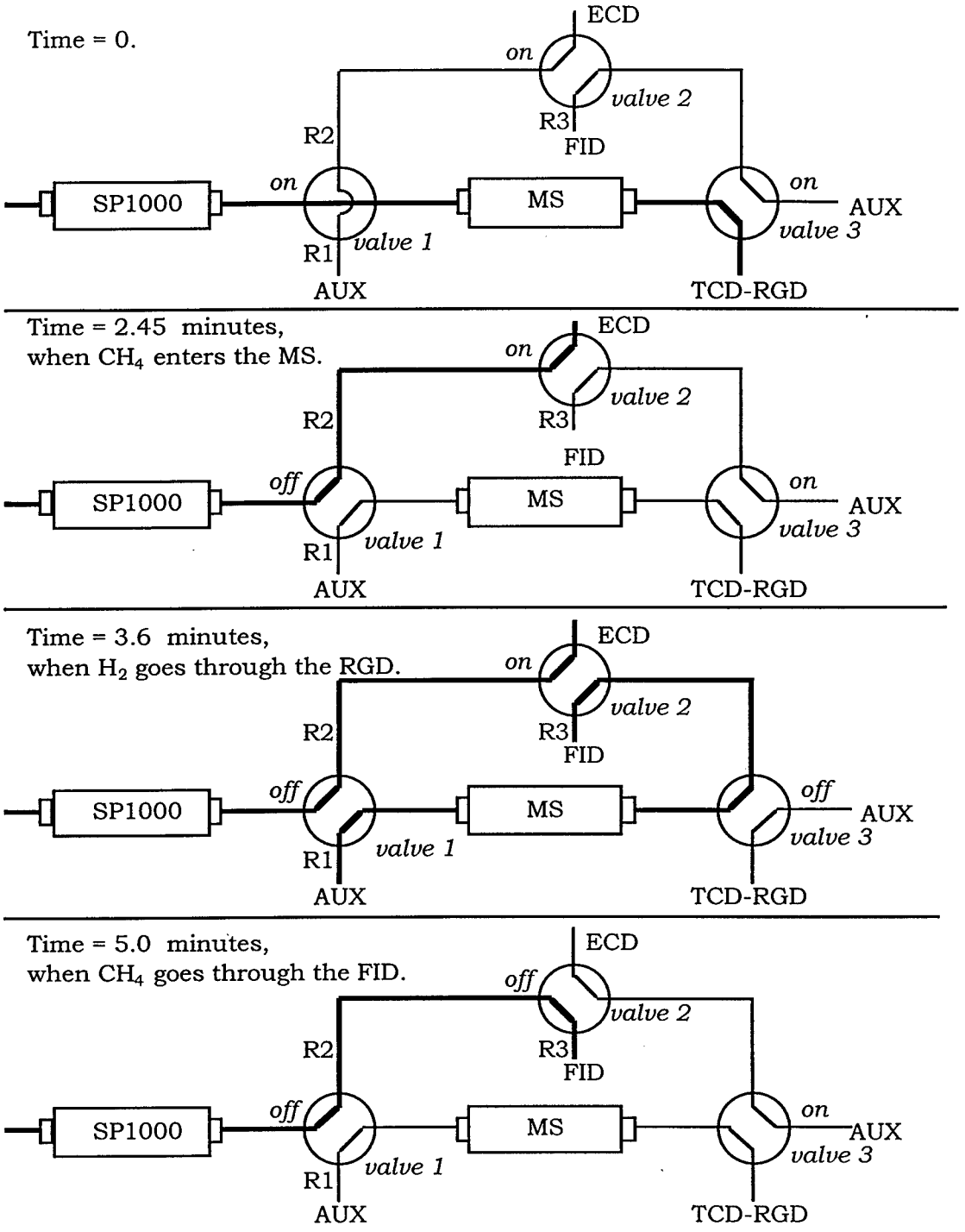


Figure 3.2 -- Diagram of the GC method for DCB, MCB, CH_4 and H_2 analysis. Where R1-R3 are restrictors 1-3 and AUX are the auxiliary flows and SP1000 is the Supelco column and MS is the molecular sieve column.

The oven temperature for GC 1 initially was set to 90°C. It held at 90°C for 4 minutes then ramped to 170°C at 30 ml/min. It held at 170°C for 3.33 minutes then ramped to 185°C at 30 ml/min and held at 185°C for 7.00 minutes. The oven temperature in GC 2 was set to a constant 60°C.

Since the three valves had to switch during the run, three restrictors were placed in line to imitate the resistance caused by the two columns. Restrictor one (R1) was placed between the auxiliary flow outlet and V1. Restrictor two (R2) was placed between V1 and V2. Both R1 and R2 were inside GC 1. Restrictor three (R3) was placed outside the GCs, between V2 and the FID.

3.G.4a Water Calibration The DCBs, MCB, benzene, CH_4 and H_2 were first calibrated in 100 ml of water in a 160-ml serum bottle. Known but different volumes of DCBs and MCB were added from their respective methanol stocks. Duplicates of each calibration level were made. These serum bottles were allowed to equilibrate on a wrist-action shaker over night and then the CH_4 and H_2 were added two hours before analysis. Two 0.5-ml headspace injections were made for each serum bottle.

3.G.4b Microcosm Calibration for Low DCB Bottles Since microcosms from different sites had slightly different headspace volumes due to the difference in densities of the soils and because the sorptive properties of the soils differ, calibrations had to be made for each microcosm set. The method of standard additions was employed, which also allowed for the determination of initial masses of DCBs, MCB and benzene in the unamended microcosms.

The DCBs and MCB were calibrated for P, R-1-s and R 1-d microcosms by preparing triplicate microcosms (S1 - S3) for each site and autoclaving them. Known (but different) amounts of the DCB water stock and MCB and benzene methanol stocks were added to S2 and S3. Nothing was added to S1. These bottles were equilibrated for 24 hours on a wrist-action shaker. Duplicate 0.5-ml headspace injections from each bottle were made and the peak area versus mass DCB or MCB added was plotted. The slope of this line was used to determine the original mass of each chemical present using the following equation:

$$\text{original mass present} = \frac{y - \text{intercept}}{\text{slope}} \qquad (2)$$

The calibration was completed by adding this original mass to each mass added and plotting against the peak area.

CH_4 and H_2 were calibrated in a water system since these gases are not sorptive and relatively insoluble in water; therefore they almost totally exist in the gas phase. Enough water was added to a three 160 ml serum bottle (W1 - W3) to give the same headspace as in the microcosms. Since the FID became swamped at CH_4 concentrations around 0.1 ml per bottle, separate low and high methane standards had to be made. Various amounts of CH_4 and H_2 were added to W1 and W3 and equilibrated for two hours on a wrist-action shaker.

3.G.4c Microcosm Calibration for High DCB Bottles For K, L, R-2 and R-LF microcosms, neat DCBs and MCB were added. Again triplicates (S1 - S3) were prepared for each site and autoclaved. S2

and S3 were uncapped and the headspace purged with N_2 while known (but different) amounts of 1,4-DCB were added. The bottles were then recapped and crimped and the remaining neat DCBs and MCB were added by syringe. These standards were equilibrated for a week on a wrist action-shaker. The calibration proceeded identically to the low DCB calibration method from here on. CH_4 and H_2 were calibrated identically to low DCB bottles.

3.G.4d Microcosm Calibration for Bottles with High MCB Production Since the FID became swamped for MCB for active microcosms, separate calibrations for high MCB concentrations had to be made. The system was calibrated identically to Section 3.G.4b except that 0.1 ml of the headspace was injected onto the GC system.

3.G.5 Volatile Acid Analysis

VFAs (acetic, propionic, isobutyric, butyric, valeric, isovaleric, and hexanoic acids) were analyzed with a FID and a 0.53-mm X 15-m Nukol® capillary column (Supelco, Inc.) on a Perkin Elmer Autosystem gas chromatograph. Samples were prepared by withdrawing 0.25 ml of liquid from the microcosms and filtering it though a Acrodisc 0.2-µm syringe filter (GelmanSciences) into a 0.25-ml glass insert (Kimble) within a 1.8-ml glass vial (Kimble). In addition, 15 µL of 1N H_3PO_4 was added to the vials from samples with low alkalinity and 15 µL of 8N H_3PO_4 was added to the vials from samples with high alkalinity. The vials were sealed with an

aluminum seal with a PTFE/red rubber liner (Kimble). An autosampler injected 0.5 µl from the vials onto the column.

The oven started out at 90°C and at seven minutes ramped to 110°C at 20 ml/min. The total run time was 18 minutes. The air and H_2 flows were identical to the conditions stated in Section 3.G.4. The N_2 flow was set to 10 ml/min.

The Autosystem was calibrated by first making a stock solution of the VFAs by adding known amounts of the VFAs to 1 L of distilled water. Then known amounts of the stock solution were delivered to 100-ml volumetric flasks and filled almost to the mark with distilled water. The solution was acidified to a pH of one or two by addition of 8 N H_3PO_4. The flask was then filled to the mark with distilled water. These standards were then transferred into the 1.8-ml glass vials and injected into the GC. The system was calibrated by plotting peak area versus mass added.

3.G.6 pH Analysis

The pH of the microcosms were approximated using pH paper (Baxter Diagnostic Inc., pH range = 4.5 -10 sensitivity = 0.5). In addition, whenever a 8 ml of DCB stock was added to a microcosm, 8 ml of liquid had to be removed. The pH of these microcosms was more accurately determined from this 8-ml sample using a Gel-Filled pH probe (Accumet). A micro flow-through pH probe (Cole Parmer) was also used to attain a more accurate pH measure. Periodically, 0.25 ml of the microcosm liquid was passed through a 0.2-µm filter and then passed through the probe. A pH measurement was also

taken during alkalinity titrations. For the soil-groundwater titration a rugged bulb probe (Accumet) was used and for the groundwater titration the Gel-filled probe was used.

3.G.7 Alkalinity Analysis

3.G.7a Alkalinity Analysis of the Soil-groundwater System

The alkalinity of the soil groundwater system was determined by adding the same amount of groundwater and soil to a wide-mouthed flask as existed in the microcosms. An initial pH reading was taken and then 0.1 N H_2SO_4 was slowly added to the flask while being continuously bubbled with air and stirred with a magnetic stir bar. The H_2SO_4 was added at about 1 to 2 ml per hour. The endpoint of the titration was the point at which water was in equilibrium with the air or a pH of 5.5. The titration only accounted for buffering by the carbonate system, because the organic acids had a pKa lower than 5.5. The stronger acid was used so that the added acid would not account for a significant amount of the liquid volume.

3.G.7b Alkalinity of the Groundwater The alkalinity of the groundwater was determined by assuming a closed system. A mass of groundwater equal to that in the microcosms was added to a flask and the initial pH was determined. Then 0.1 N H_2SO_4 was added quickly until a pH of 4.5 was reached. A pH_{CO2} of 4.5 is the endpoint for a alkalinity titration when there is an alkalinity of approximately 150 mg/L as $CaCO_3$.

3.G.8 Conductivity Analysis

The conductivity of the groundwater was measured by placing a conductivity probe (Fisher Scientific) in 100 ml of groundwater.

CHAPTER FOUR -- RESULTS

4.A Plattsburgh Air Force Base

The P microcosms were prepared on April 8, 1998. Table 4.1 contains the sample data.

Table 4.1 -- P Sample Data

Moisture Content (% of wet weight)	17.27
Groundwater Alkalinity (meq/L)	11.2
Groundwater + Soil Alkalinity (meq/L)*	48
Conductivity (mS/cm)	592
Groundwater + Soil pH*	7.5

* At soil/water ratio used in microcosms

Fifteen microcosms were prepared for this site. Three were used for calibration purposes, and from the calibration the background concentrations of DCBs at the site were determined. The remaining 12 microcosms were monitored throughout the experiment. Microcosms #1 through #9 were made with 50 g of soil and 46 g of groundwater and DCBs (4 g of the DCB stock 3.G.1a). The total levels of DCBs in the microcosms were the sums of the background and the added levels (Table 4.2).

Table 4.2 -- P MCB and DCB Data

	Background (µmole)	Added (µmole)	Total (µmole)
MCB	0	0	0
1,3-DCB	0.02	0.44	0.46
1,4-DCB	0.04	0.43	0.47
1,2-DCB	0.05	0.59	0.64

In addition, microcosms #1 through #4 received 100 mg/L YE (0.2 ml from the YE stock 3.G.1c) and microcosms #8 and #9 were autoclaved. Microcosms #10 through #12 were made with 100 ml of distilled water, DCBs (8 g of the DCB stock) and were autoclaved.

The first DCB and MCB data points were taken on April 11, 1998 (time zero). All the microcosms except #4 were run for 419 days. Microcosm #4 broke and was only run for 389 days. No dechlorination was observed in any microcosm. The DCB and MCB concentration profiles for microcosms #1 through #9 were very similar and an exemplary profile of a live microcosm (#3) is shown in Figure 4.1 and an exemplary profile of an autoclaved control (#9) is shown in Figure 4.2. The DCB and MCB concentration profiles for microcosm #10 through #12 (water controls) were similar and exemplary results from microcosm #12 are shown in Figure 4.3.

Due to the observed scatter in the data before day 122, the syringe was changed from a VICI 0.25-ml syringe to a BD plastic 1-ml syringe. The decreases in DCB concentration in Figures 4.1, 4.2 and 4.3 were probably due to losses through the septum.

4.B Robins Air Force Base at Well R13-2W

The R-2 microcosms were prepared on October 23, 1998. Table 4.3 contains the sample data.

Fifteen microcosms were prepared for this site. Three were used for calibration purposes, and from the calibration the background concentrations of DCBs at the site were determined. The remaining 12 microcosms were monitored throughout the

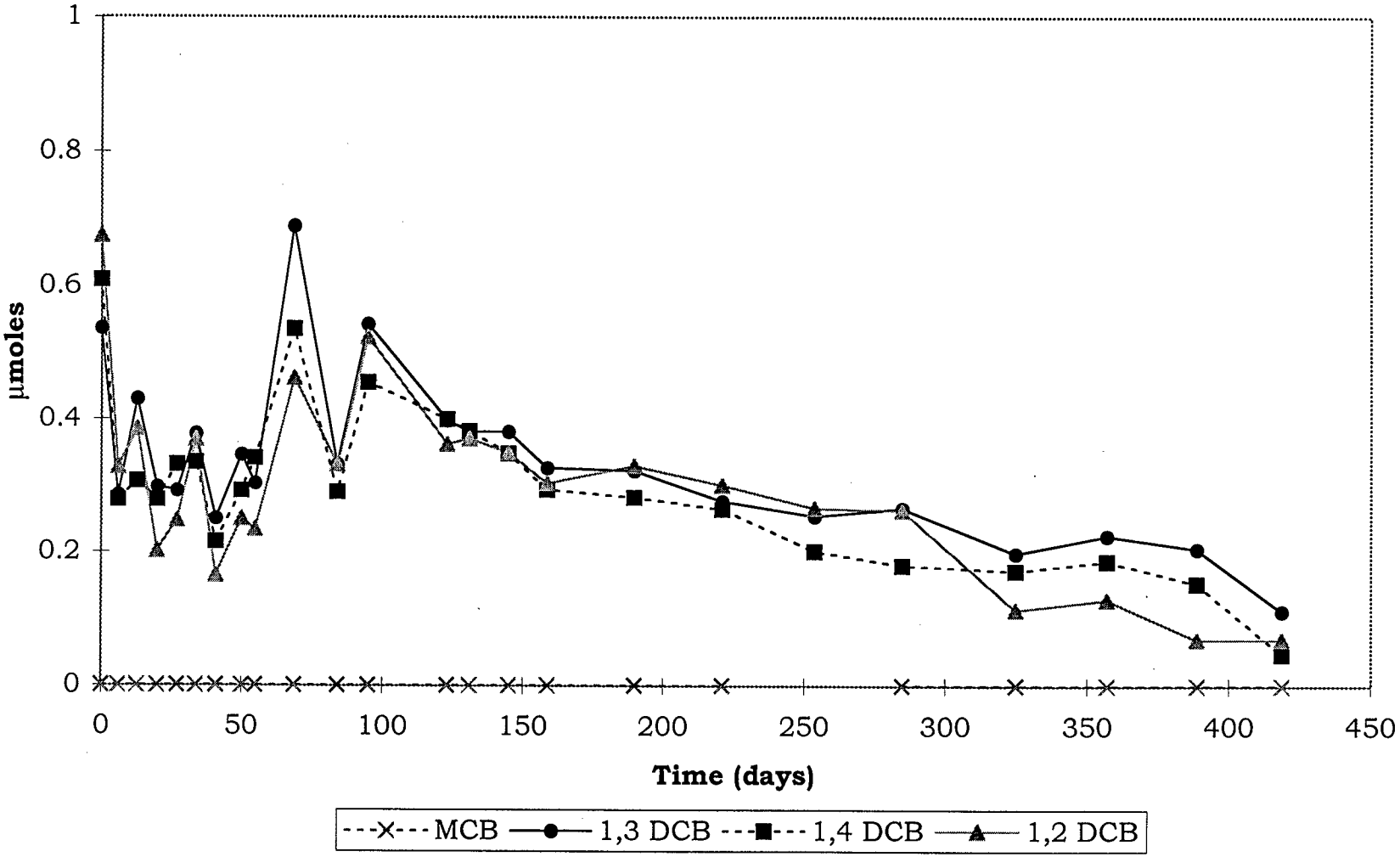


Figure 4.1 -- DCB and MCB Concentrations in P Microcosm #3 (fed)

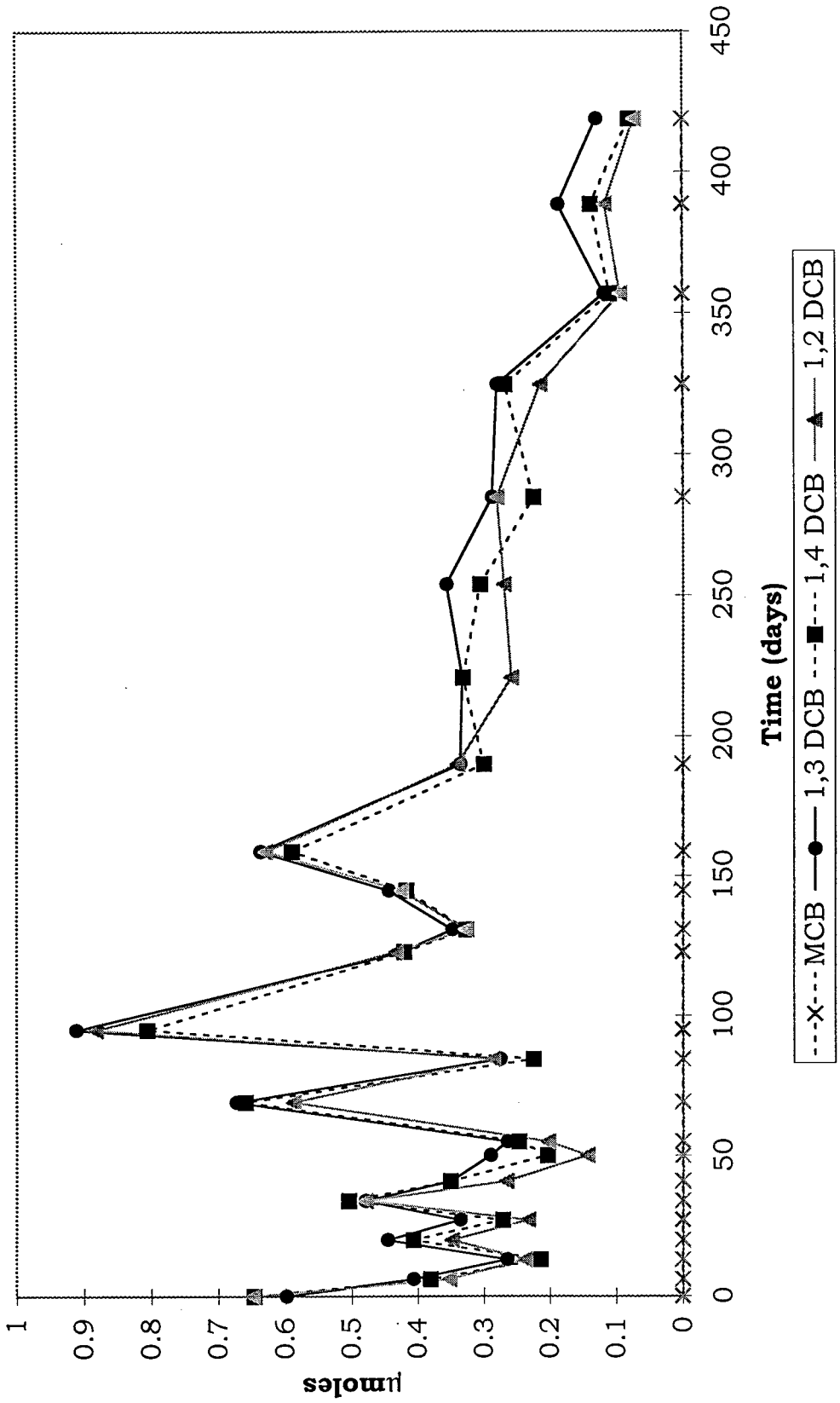


Figure 4.2 -- DCB and MCB Concentrations in P Microcosm #9 (autoclaved control)

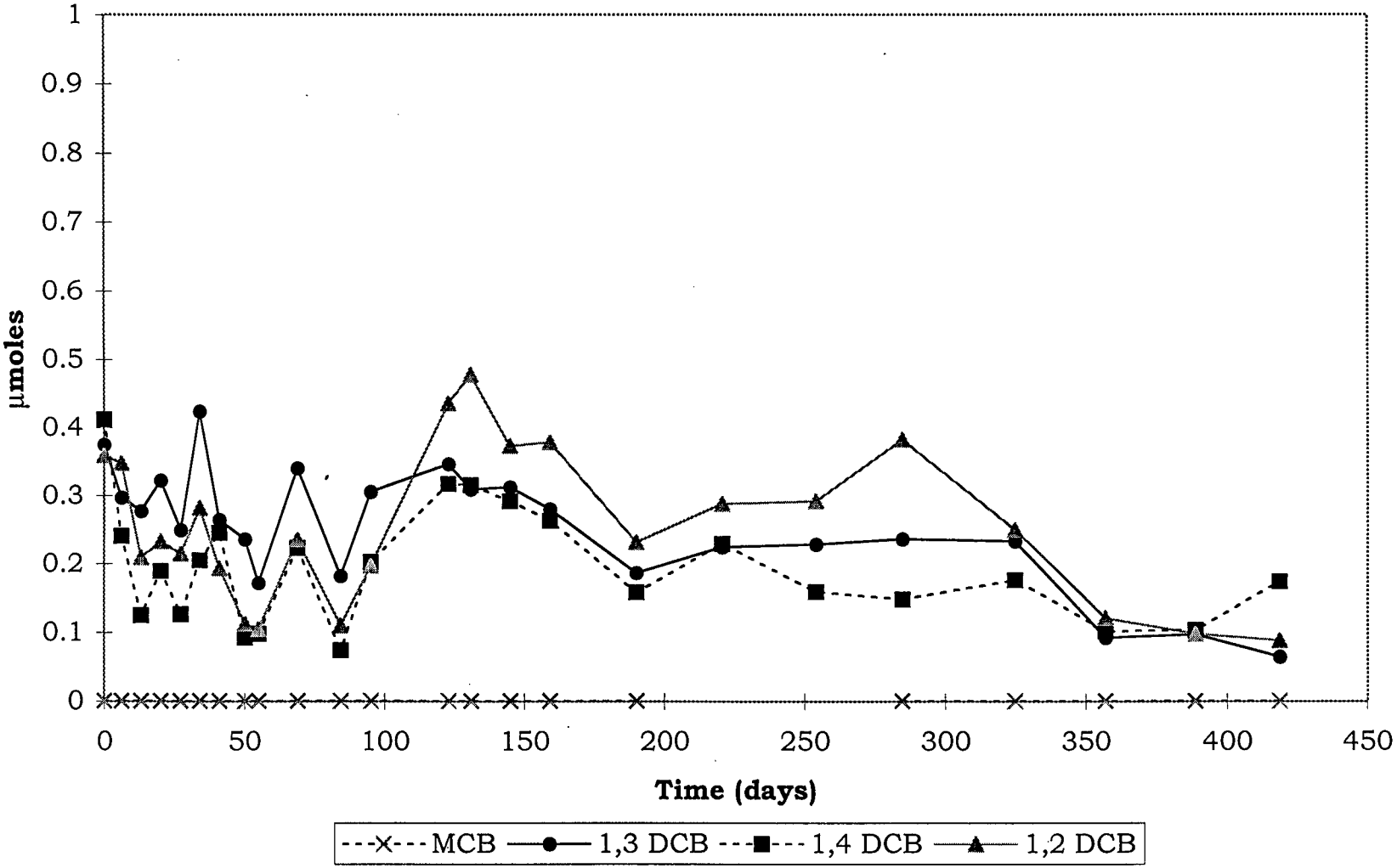


Figure 4.3 -- DCB and MCB Concentrations in P Microcosm #12 (Water-control)

Table 4.3 -- R-2 Sample Data

Moisture Content (% of wet weight)	20.84
Groundwater Alkalinity (meq/L)	<0.25
Groundwater + Soil Alkalinity (meq/L)*	<0.25
Conductivity (mS/cm)	64.7
Groundwater + Soil pH*	5.49

* At soil/water ratio used in microcosms

experiment. Microcosms #1 through #9 were made with 50 g of soil and 100 g of groundwater and DCBs (2.0 µl of neat 1,2-DCB, 2.3 µl of neat 1,3-DCB and 3 mg of neat 1,4-DCB). The DCBs were added on day zero. The total levels of DCBs in the microcosms were the sums of the background and the added levels (Table 4.4).

Table 4.4 -- R-2 MCB and DCB Data

	Background (µmole)	Added (µmole)	Total (µmole)
MCB	22.70	0	22.7
1,3-DCB	0	20.29	20.29
1,4-DCB	0	20.55	20.55
1,2-DCB	1.62	17.89	19.51

In addition, microcosms #1 through #4 received 100 mg/L YE (0.2 ml of the YE stock) and microcosms #8 and #9 were autoclaved. Microcosms #10 through #12 were made with 100 ml of distilled water, DCBs (2.3 µl neat of both 1,2- and 1,3-DCB and 3 mg of neat 1,4-DCB) and were autoclaved.

The first DCB and MCB data points were taken at day 7. All the microcosms were run for 187 days. No dechlorination was observed in any of the microcosms. All the DCB and MCB concentration profiles for microcosms #1 through #9 were similar and exemplary results from microcosm #1 are shown in Figure 4.4.

The DCB and MCB concentration profiles for water-control microcosms #10 through #12 were similar and exemplary results from microcosm #11 are shown in Figure 4.5.

4.C Robins Air Force Base Landfill Leachate

The R-LF microcosms were prepared on October 22, 1998. Table 4.5 contains the sample data.

Table 4.5 -- R-LF Sample Data

Leachate Alkalinity (meq/L)	6.75
Conductivity (mS/cm)	984
Leachate pH	6.16

Fifteen microcosms were prepared for this site. Three were used for calibration purposes, and from the calibration the background concentrations of DCBs and MCB at the site were determined. The remaining 12 microcosms were monitored throughout the experiment. Microcosms #1 through #9 were made with 100 g of leachate and DCBs (1.6 µl of neat 1,3-DCB and 2.3 mg of neat 1,4- DCB). The DCBs were added at day zero. The total levels of DCBs in the microcosms were the sums of the background and the added levels (Table 4.6).

Table 4.6 -- R-LF MCB and DCB Data

	Background (µmole)	Added (µmole)	Total (µmole)
MCB	17.61	0	17.61
1,3-DCB	6.72	14.12	20.84
1,4-DCB	5.13	15.75	20.88
1,2-DCB	27.29	0	27.29

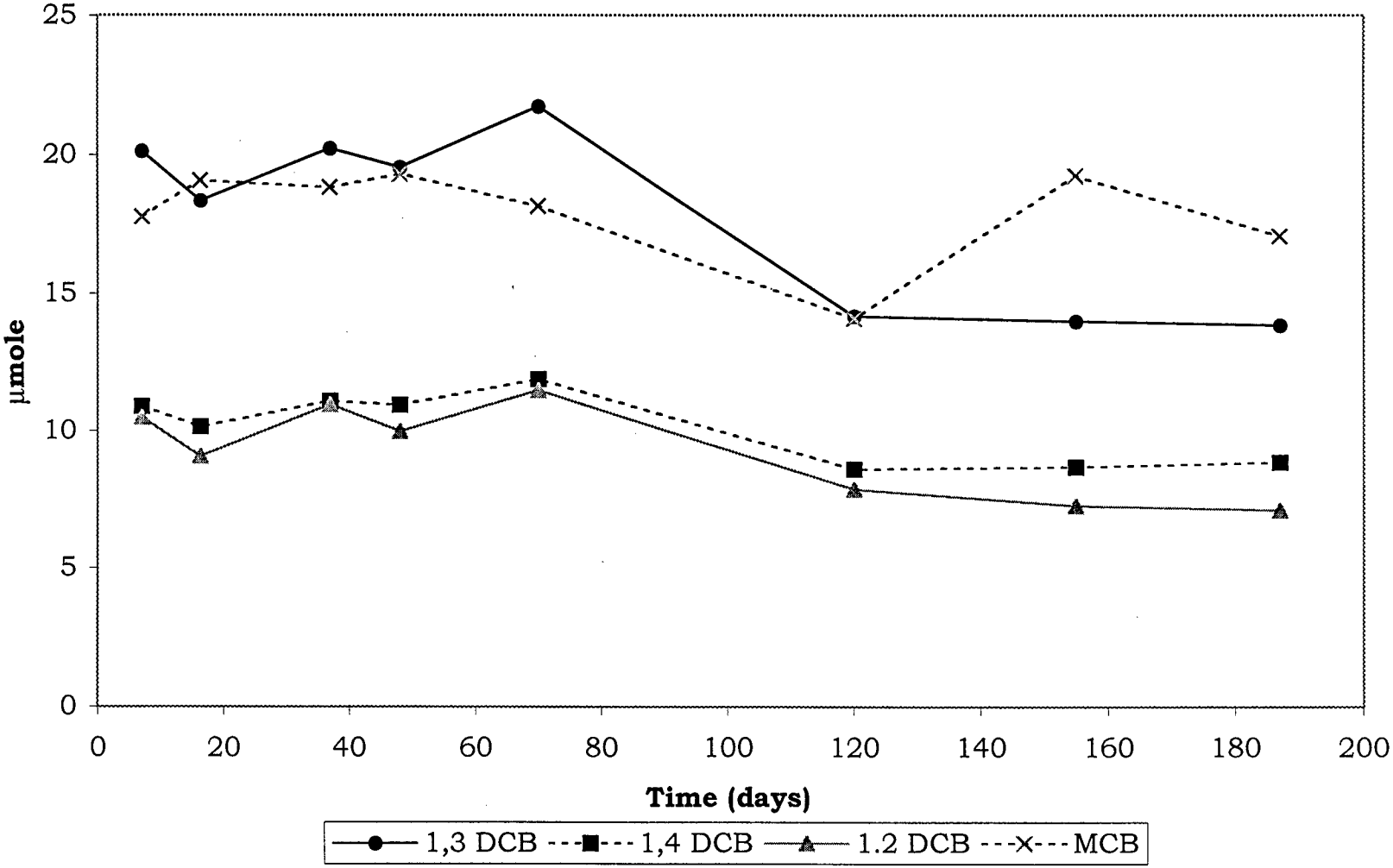


Figure 4.4 -- DCB and MCB Concentrations in R-2 Microcosm #1 (Fed)

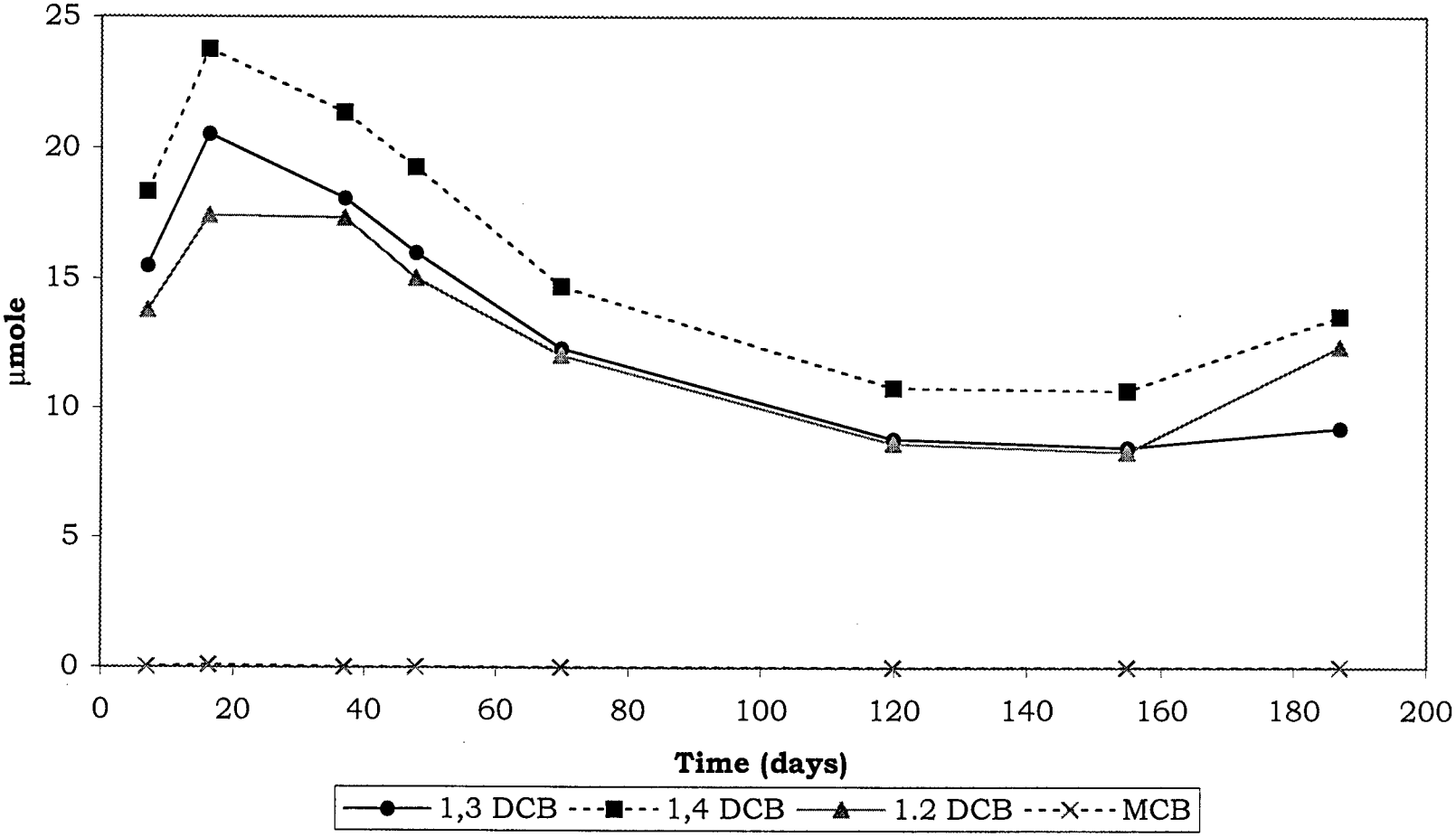


Figure 4.5 -- DCB and MCB Concentrations in R-2 Microcosm #11, (Water-control)

In addition, microcosms #1 through #4 received 100 mg/L YE (0.2 ml from the YE stock) and microcosms #8 and #9 were autoclaved. Microcosms #10 through #12 were made with 100 ml of distilled water, DCBs (2.3 µl neat of both 1,2- and 1,3-DCB and 3 mg of neat 1,4-DCB) and were autoclaved.

The first DCB and MCB data points were taken on day 7. All the microcosms were run for 194 days. No dechlorination was observed in any microcosm. All the DCB and MCB concentration profiles for microcosm #1 through #9 were similar and exemplary results from microcosm #4 are shown in Figure 4.6. The DCB and MCB concentration profiles for the water-control microcosms #10 through #12 were similar to Figure 4.5.

4.D Digested Sludge From the Ithaca Wastewater Treatment Plant

The DS microcosms were prepared on June 28, 1998. The moisture content of the sludge was 97.49%. Ten microcosms were made for this site. Three were used for calibration purposes. There were no background concentrations of DCBs or MCB. The remaining seven microcosms were monitored throughout the experiment. Microcosms #1 through #5 were made with 92 g of sludge and DCBs (8 g of the DCB stock). The DCBs were added at day zero. At 71 days, after vigorous gas production had diminished, high levels of DCBs were added to microcosms #1 through #4 (2.3 µl neat of both 1,2- and 1,3-DCB and 3 mg of neat 1,4-DCB). The total levels of

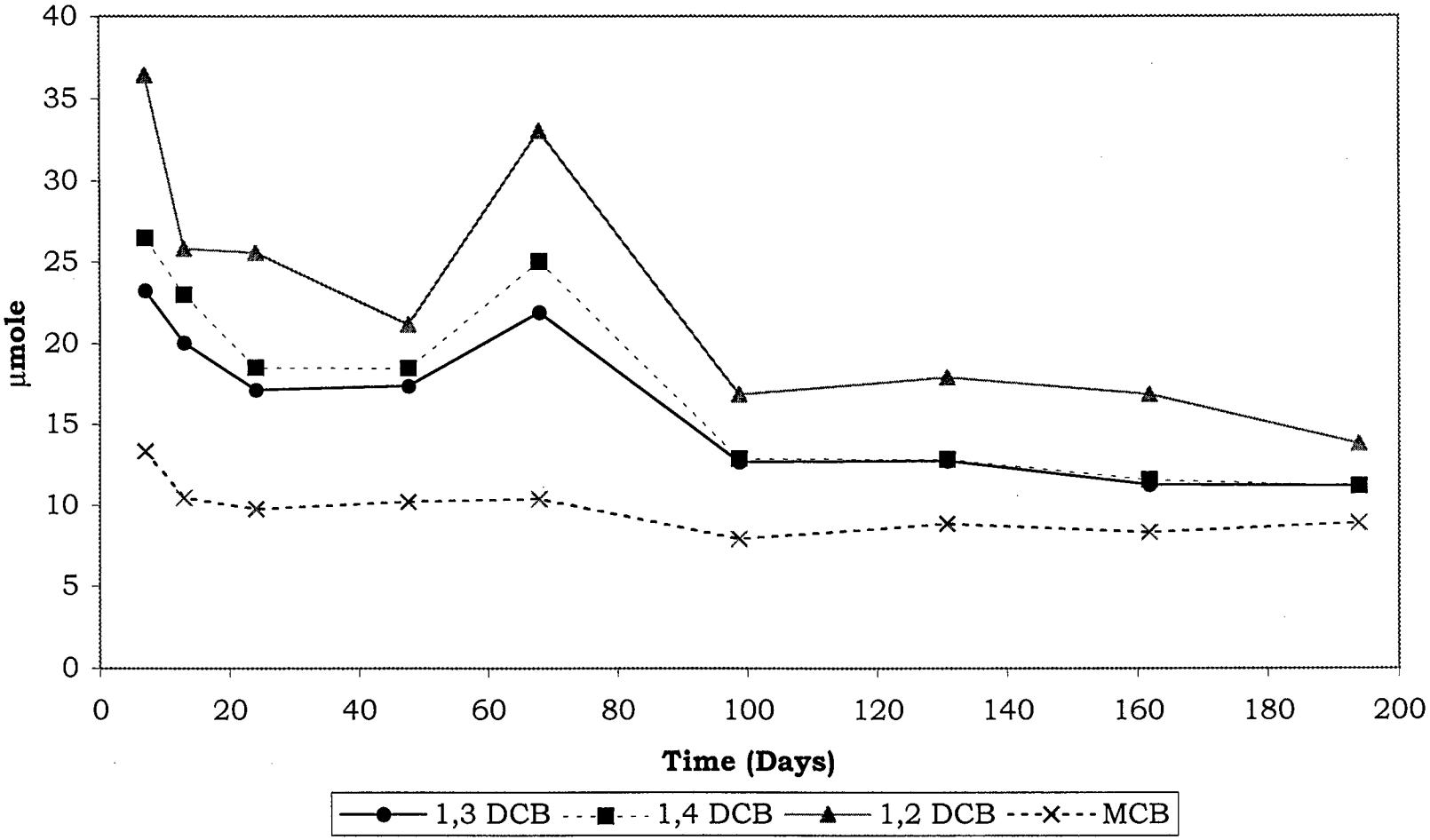


Figure 4.6 -- DCB and MCB Concentrations in R-LF Microcosm #4 (Fed)

DCBs in the microcosms were the sums of the added levels (Table 4.7).

Table 4.7 -- DS MCB and DCB Data

	First Addition to 1-7 (μmole)	Second Addition to 1-4,7 (μmole)	Total (μmole)
MCB	0	0	0
1,3-DCB	0.87	20.29	21.16
1,4-DCB	0.85	20.55	21.4
1,2-DCB	1.18	20.57	21.75

Microcosms #6 and #7 were made with 100 ml of distilled water and DCBs from the DCB stock (8 g). In addition, on day 38, microcosm #7 received 2.3 μl neat of both 1,2- and 1,3-DCB and 3 mg of neat 1,4-DCB. Microcosms #6 and #7 were both autoclaved at initial setup.

The excess pressures in microcosms #1 through #5 were relieved every few months. This was done by placing a sterile needle on a 10-ml ground glass syringe and allowing the pressure in the microcosm to push the plunger out of the barrel.

The first DCB and MCB data points were taken on day 1 for all microcosms. Microcosms #1 through #5 were run for 348 days. Microcosms #6 and #7 were run for 316 days. Slow dechlorination was observed in microcosms #1 through #5, with the highest rate of dechlorination in microcosm #2 (Figure 4.7). 1,2-DCB was the only DCB isomer degraded so far and the MCB production accounted for 10% of the total DCBs (added plus initial). However, because of losses over prolonged incubation, this can suggest a misleadingly low conversion. Perhaps a better way to look at conversion to MCB, is as a percentage of total recovered chlorinated benzenes. For

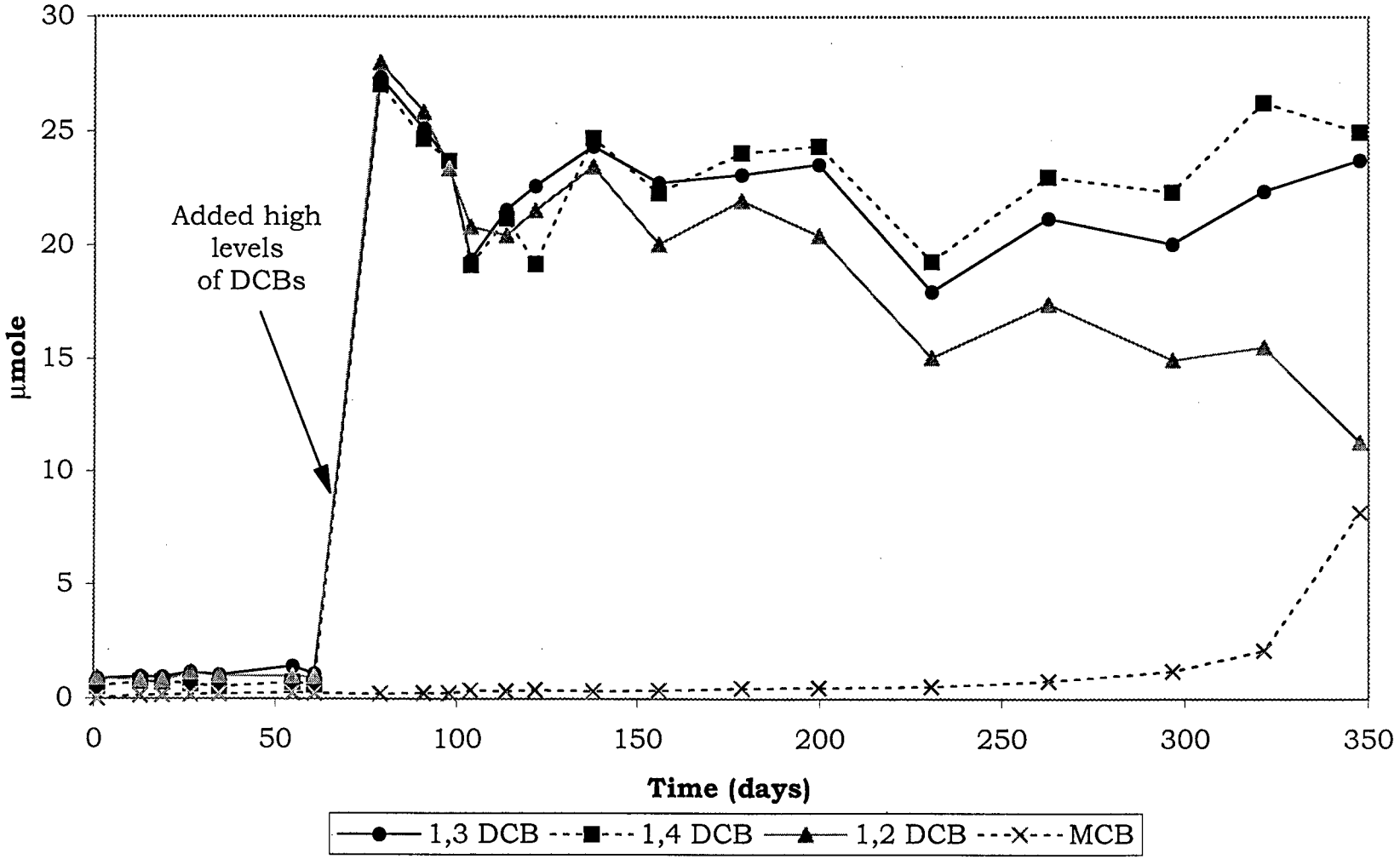


Figure 4.7 -- DCB and MCB Concentrations for DS Microcosm #2

microcosm #2, the MCB production accounted for 12% of the total recovered chlorinated benzenes.

Although the DCB profile for water-control microcosms #6 and #7 differed because of the different levels of DCBs added to each microcosm, no dechlorination was observed in either. The DCB and MCB concentration profile for microcosm #6 was similar to Figure 4.2. The DCB and MCB concentration profile for microcosm #7 was similar to Figure 4.5 after the addition of the neat DCBs.

4.E Kelly Air Force Base

The K microcosms were prepared on June 2, 1998. Three microcosms (#2, #3 and #6) broke during the experiment and were remade on October 27, 1998. Table 4.8 contains the sample data.

Table 4.8 -- K Sample Data

Moisture Content (% of wet weight)	18.66
Groundwater Alkalinity (meq/L)	7
Groundwater + Soil Alkalinity (meq/L)*	>100
Conductivity (mS/cm)	578
Groundwater + Soil pH*	7.16

* At soil/water ratios used in microcosms

Fifteen microcosms were made for this site. Three were used for calibration purposes, and from the calibration the original concentrations of DCBs at the site were determined. The remaining 12 microcosms were monitored throughout the experiment. Microcosms #1 through #9 were made with 50 g of soil and 104 g of groundwater and DCBs (2.0 µl of neat 1,2-DCB, 2.3 µl of neat 1,3-DCB and 3 mg of neat 1,4-DCB). Due to the high native MCB

concentration and the presence of a LNAPL phase, the microcosms were purged with N_2/CO_2 for about eight hours. The DCBs were added on day zero. The total levels of DCBs in the microcosms were the sums of the background (after purging) and the added levels (Table 4.9).

Table 4.9 -- K MCB and DCB Data

	Background (µmole)	Added (µmole)	Total (µmole)
MCB	3.78	0	3.78
1,3-DCB	0	20.29	20.29
1,4-DCB	4.51	20.41	24.92
1,2-DCB	0	20.57	20.57

In addition, microcosms #1 through #4 received 100 mg/L YE (0.2 ml from the YE stock) and microcosms #8 and #9 were autoclaved. Microcosms #10 through #12 were made with 100 ml of distilled water, DCBs (2.3 µl neat of both 1,2- and 1,3-DCB and 3 mg of neat 1,4-DCB) and were autoclaved.

The first DCB and MCB data points were taken on day 7. On day 163 after 1,2- and 1,3-DCB had been consumed in microcosm #1, 1,2-, 1,3-, and 1,4-DCB were added (20.57, 20.29 and 20.41 µmoles). YE (0.2 ml from the YE stock) was again added to microcosm #1 on day 173. Microcosm #1 developed a hair-line fracture and was only run for 220 days (Figure 4.8). The MCB production accounted for 58% of the total (initial plus added) DCBs (but 82% of the total recovered chlorinated benzenes).

On day 225, DCBs (20.29 µmole of 1,3-DCB, 20.41 µmole of 1,4-DCB and 20.57 µmole of 1,2-DCB) were added to microcosm #4. YE.

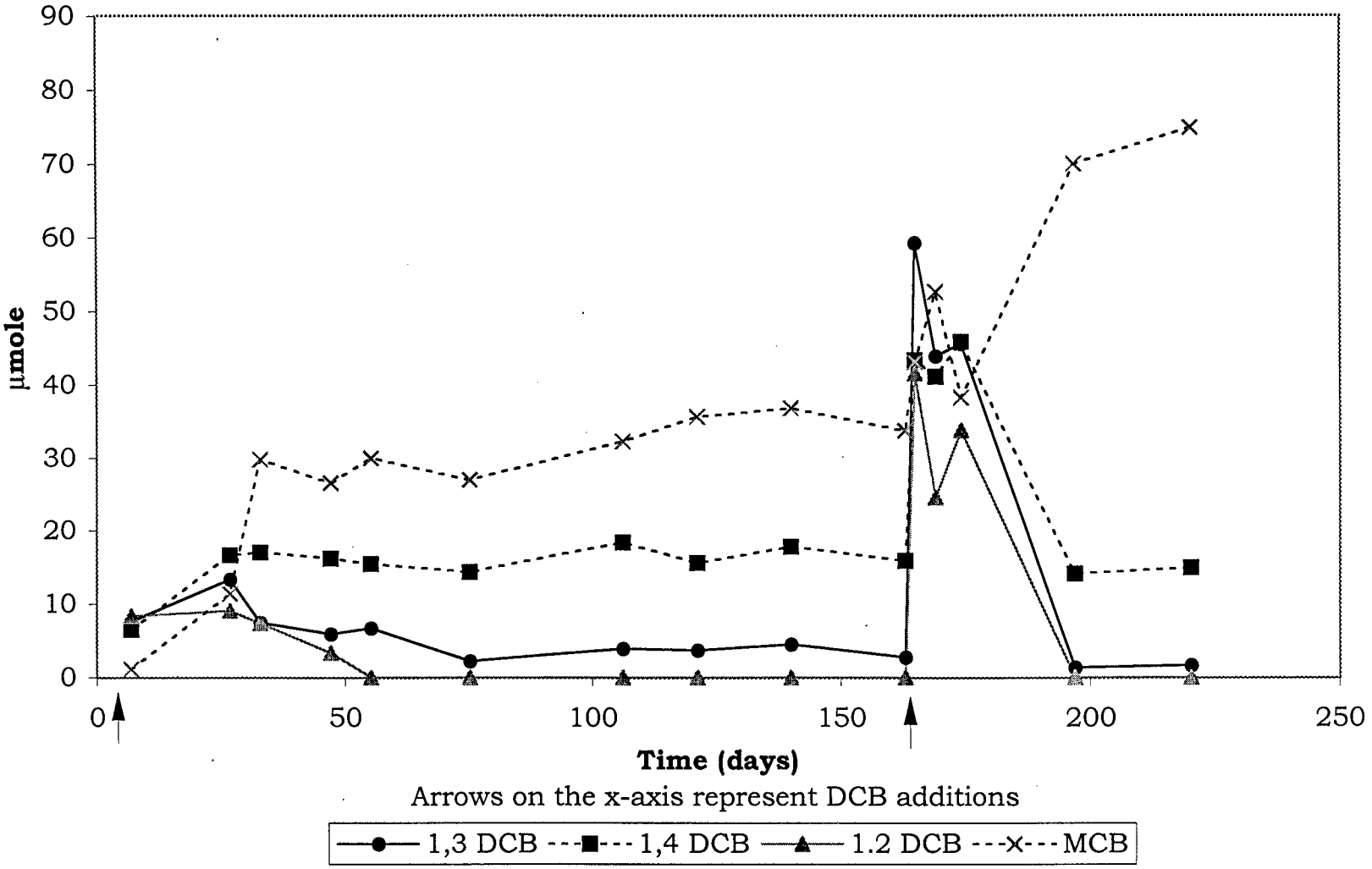


Figure 4.8 -- DCB and MCB Concentrations for K Microcosm #1 (Fed)

(0.2 ml of the YE stock) was again added to microcosm #4 on day 226. Microcosm #4 (Figure 4.9) was run for 282 days. The MCB production accounted for 52% of the total (added plus initial) DCBs (but 77% of the total recovered chlorinated benzenes). Microcosm #3 was also run for 282 days

The DCB and MCB concentration profiles for unfed, microcosms #5 and #7 were similar and exemplary results from microcosm #5 are shown in Figure 4.10. The MCB production within microcosm #5 accounted for 57% of the total (initial plus added) DCBs (but 71% of the total recovered chlorinated benzenes).

Microcosms #2, #3 and #6 were run for 242 days. The DCB and MCB concentration profiles for #2 (fed), #3 (fed) and #6 (unfed) were similar and exemplary results from microcosm #6 are shown in Figure 4.11.

Dechlorination was observed in all soil microcosms (including the autoclaved controls). After it was evident that the two controls were not sterile, these microcosms were autoclaved again at day 200. This seemed to stop the dechlorination in the two controls. The DCB The DCB and MCB concentration profiles for the autoclaved soil control microcosms (#8 and #9) were similar to each other and Figure 4.12 contains the concentration profile for microcosm #8.

The DCB and MCB concentration profiles for water-control microcosms #10 through #12 were similar to Figure 4.5.

On May 3, 1999, three enrichments were made in basal medium. Enrichments #1 through #3 were made with 1 ml, 2 ml and 5 ml inocula from microcosm #1. Enrichments #1 through #3 also

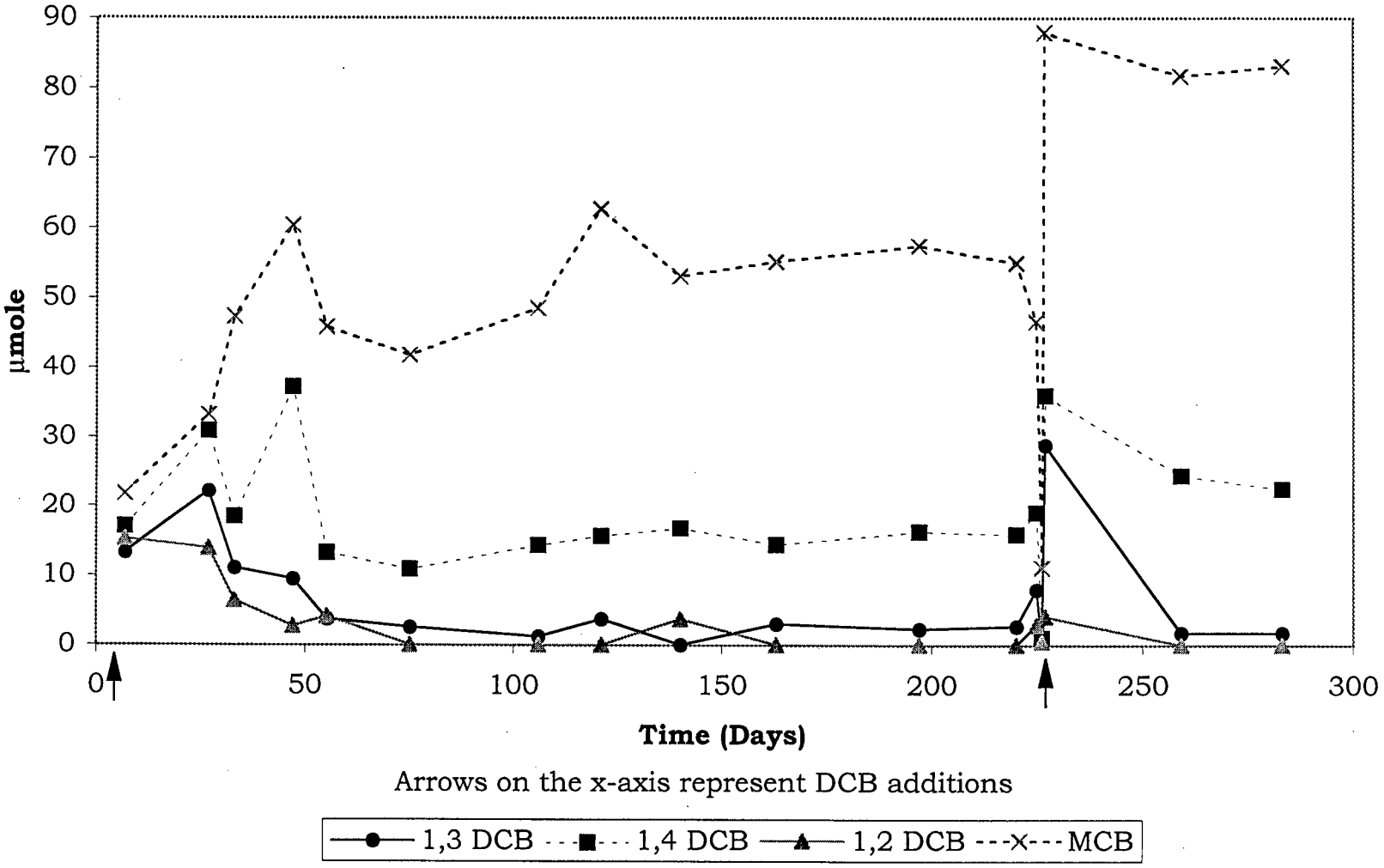


Figure 4.9 -- DCB and MCB Data for K Microcosm #4 (Fed)

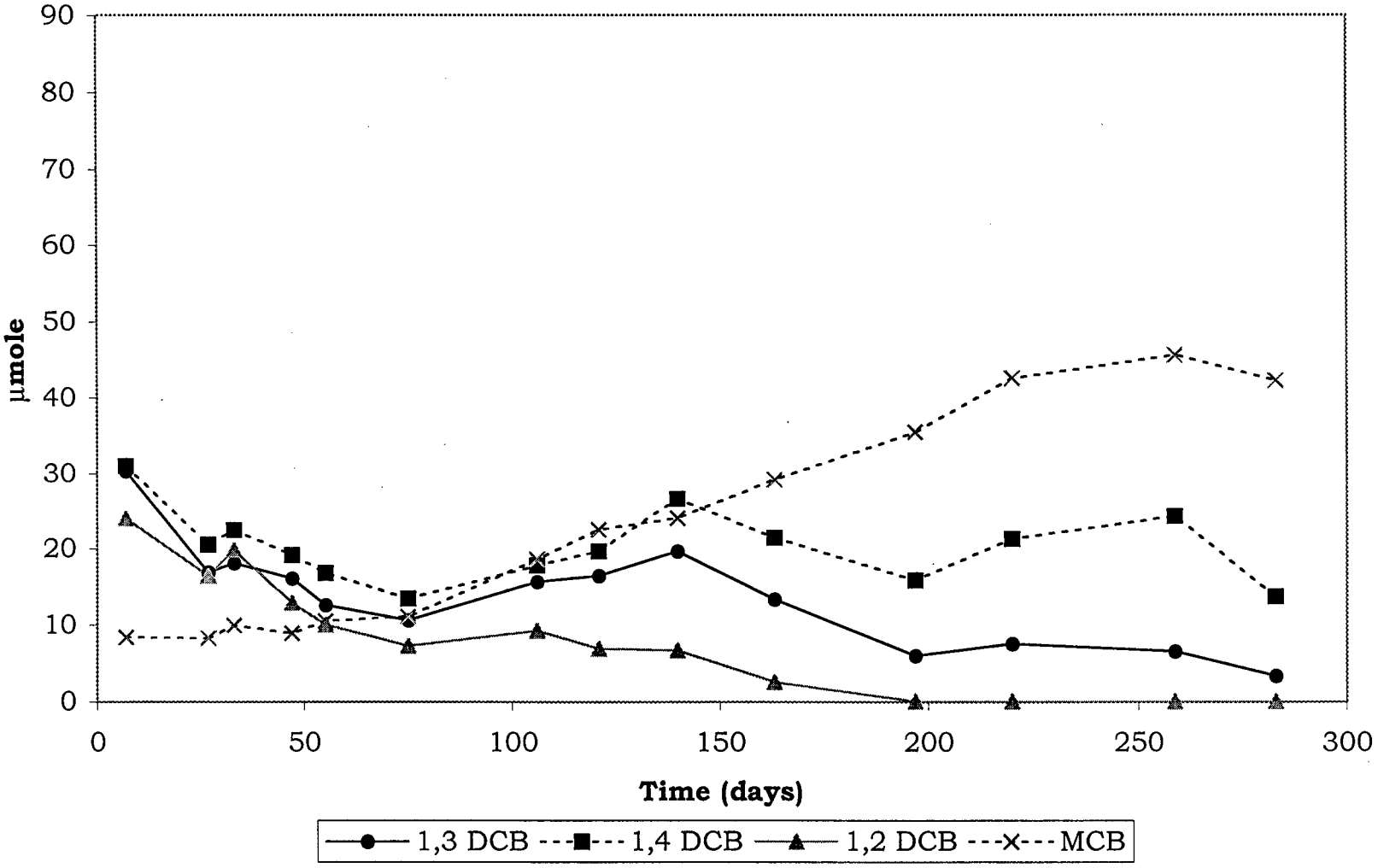


Figure 4.10 -- DCB and MCB Data for K Microcosm #5 (unfed)

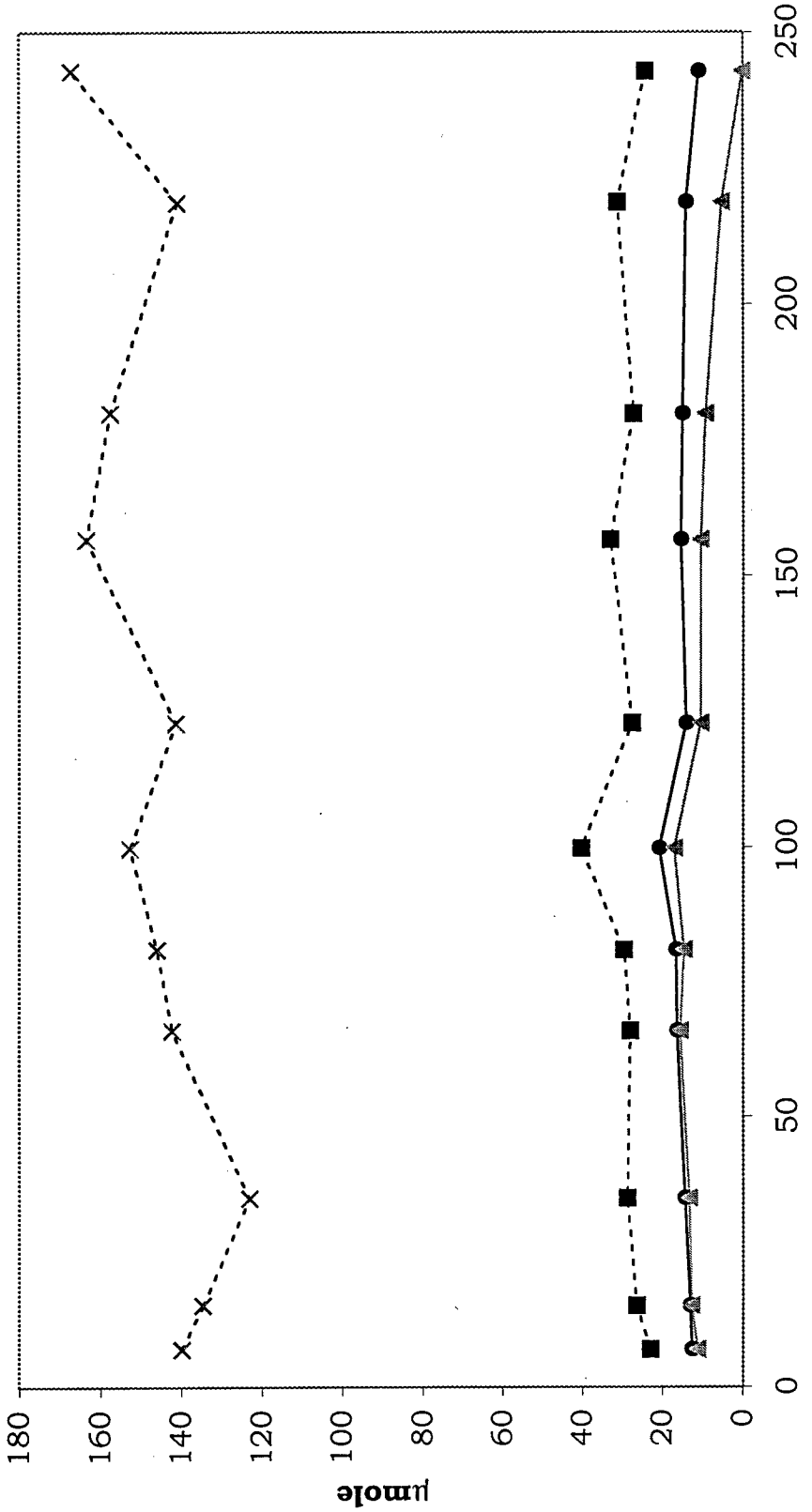


Figure 4.11 -- DCB and MCB Concentrations for K Microcosm #6, re-made (unfed)

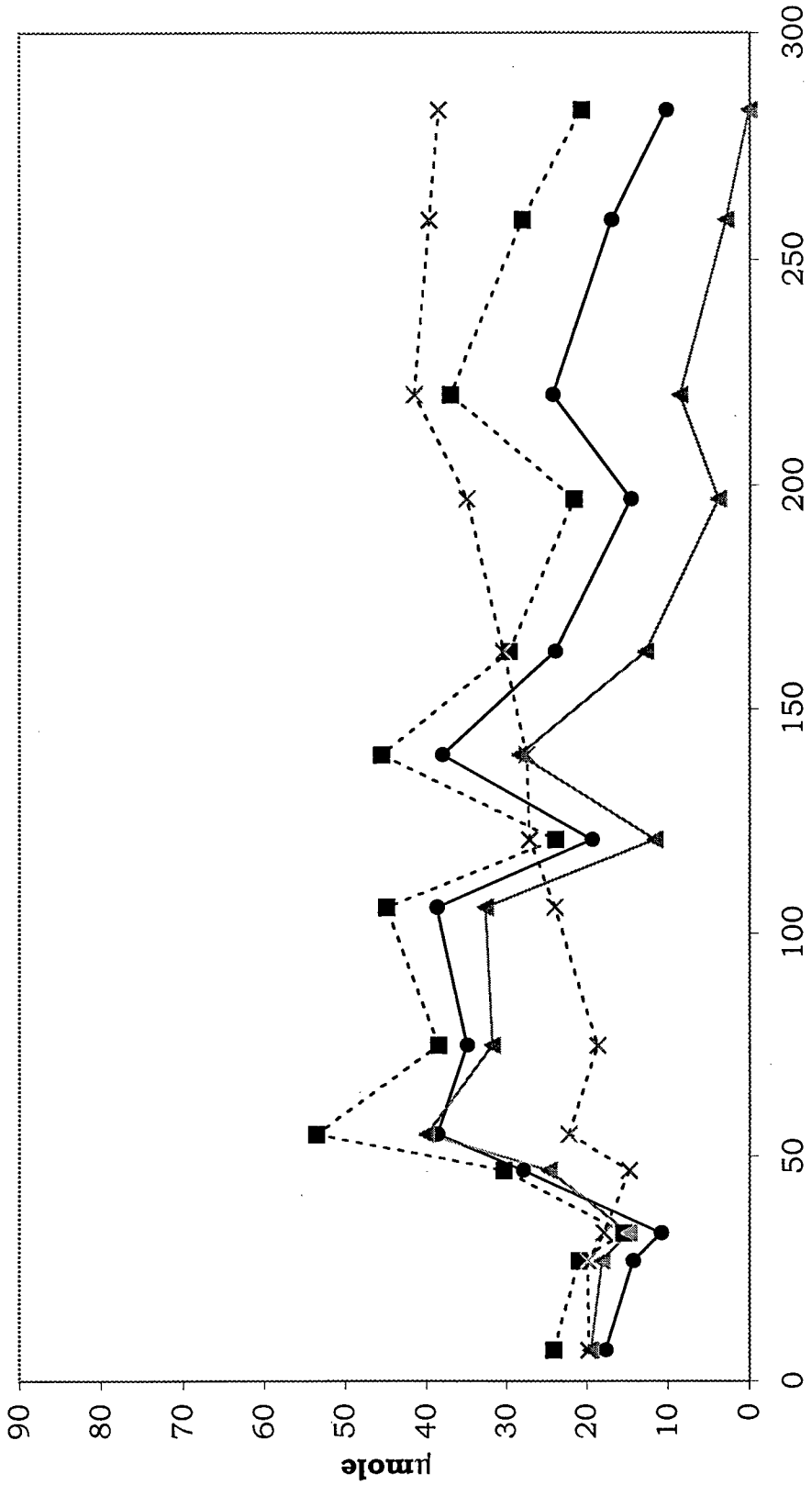


Figure 4.12 -- DCB and MCB Concentrations for K Microcosm #8 (Autoclaved control)

received neat DCBs (2.3 µl of 1,2- and 1,3-DCB and 3 mg of 1,4-DCB) and 0.2 ml of YE stock.

The first DCB and MCB data points for the enrichments were taken on day 1. All the enrichments were run for 60 days. Dechlorination was evident in all three enrichments (Figure 4.13. 4.14 and 4.15). The MCB production in enrichment #1 accounted for 14% of the total (initial plus added) DCBs (but 34% of the total recovered chlorinated benzenes). The MCB production in enrichment #2 accounted for 42% of the total (initial plus added) DCBs (but 68% of the total recovered chlorinated benzenes). The MCB production in enrichment #3 accounted for 62% of the total (initial plus added) DCBs (but 84% of the total recovered chlorinated benzenes).

In all K microcosms and enrichments, 1,2-DCB was degraded first followed by 1,3-DCB and 1,4-DCB.

4.F Louisiana Wetland Sediment

The L microcosms were prepared on August 7, 1998. The moisture content of the slurry was 73.78%.

Fifteen microcosms were prepared for this site. Three were used for calibration purposes, and from the calibration the background concentrations of DCBs and MCB at the site were determined. The remaining 12 microcosms were monitored throughout the experiment. Microcosms #1 through #9 were made by dividing the slurry between the microcosms (76 g) and then adding 30 g of basal medium and DCBs (2.0 µl neat 1,2-DCB, 2.3 µl neat 1,3-DCB and 3 mg of neat 1,4-DCB). The DCBs were added on day zero.

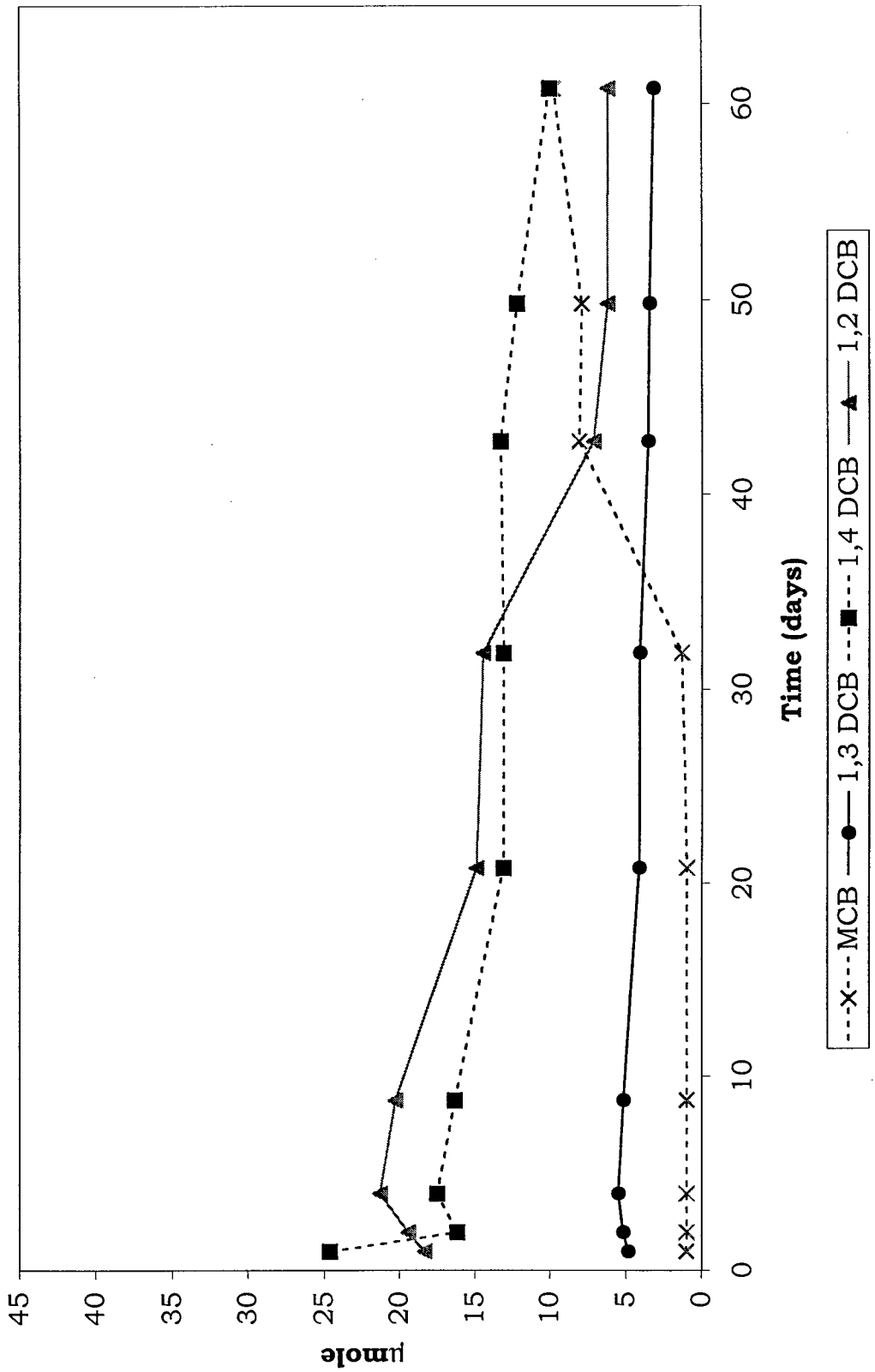


Figure 4.13 -- DCB and MCB Data for K Enrichment #1

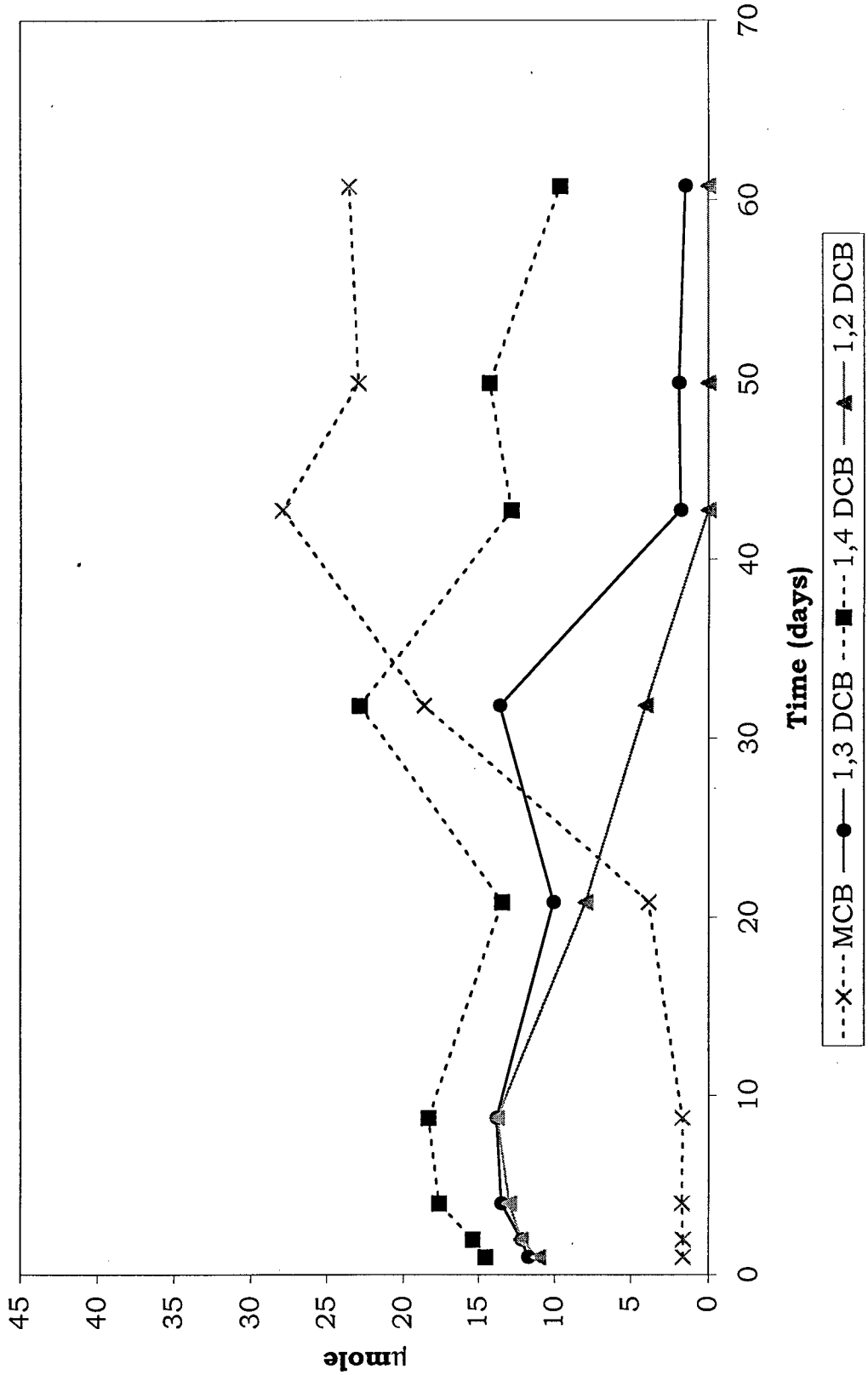


Figure 4.14 -- DCB and MCB Data for K Enrichment #2

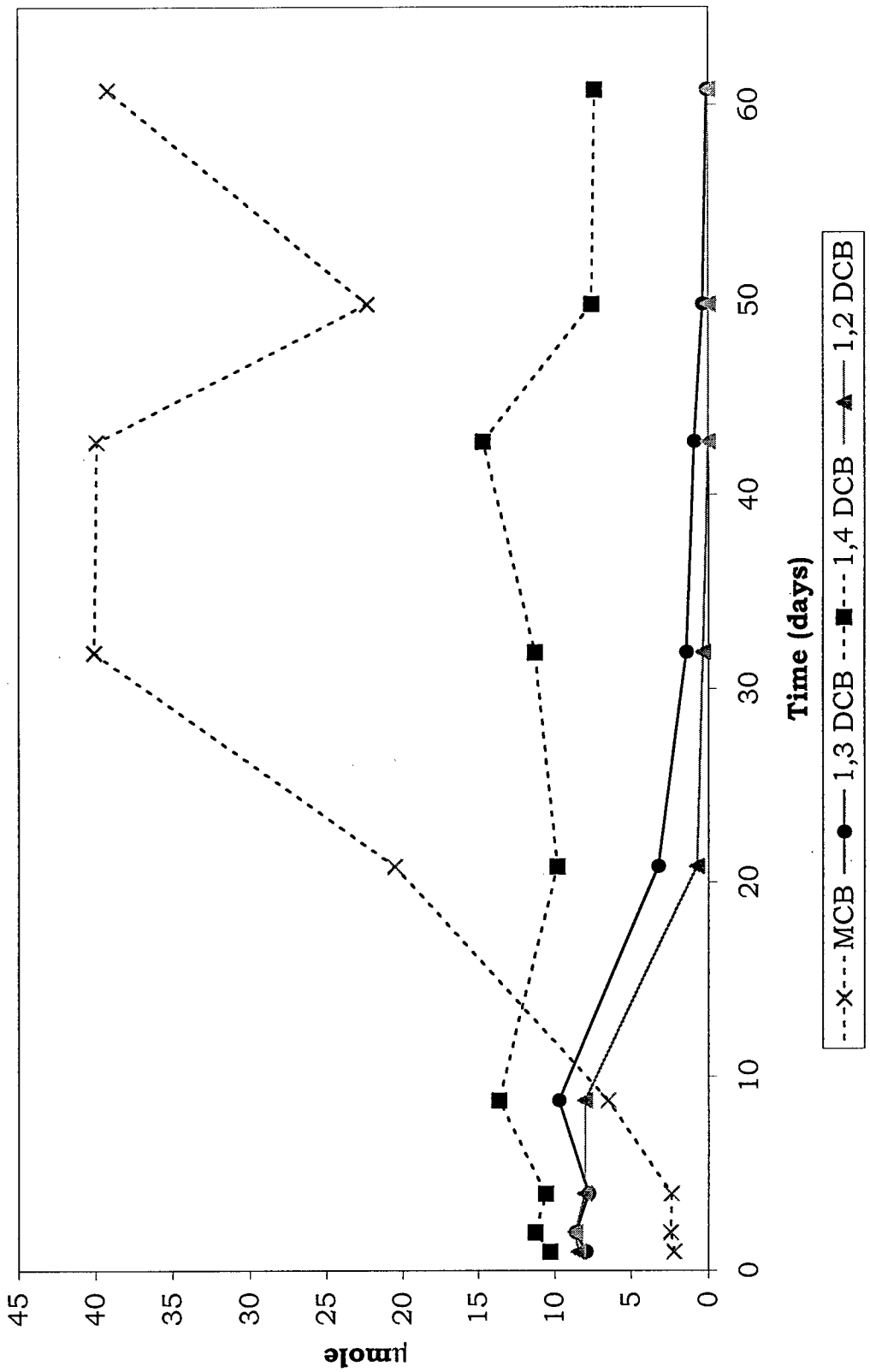


Figure 4.15 -- DCB and MCB Data for K Enrichment #3

The total levels of DCBs in the microcosms were the sums of the background and the added levels (Table 4.10).

Table 4.10 -- L MCB and DCB Data

	Background (μmole)	Added (μmole)	Total (μmole)
MCB	0.41	0	0.41
1,3-DCB	0	20.29	20.29
1,4-DCB	0	20.41	20.44
1,2-DCB	0	20.57	20.57

In addition, microcosms #1 through #4 received 100 mg/L YE (0.2 ml from the YE stock) and microcosms #8 and #9 were autoclaved. Microcosms #10 through #12 were made with 100 ml of distilled water, DCBs (2.3 μl neat of both 1,2- and 1,3-DCB and 3 mg of neat 1,4-DCB) and were autoclaved.

Around day 60, since the VFAs were low and since no H_2 had accumulated, microcosms #1 through #4 were given another dose of YE. On day 260, DCBs were added to microcosms #1 and #3 (20.29 μmole of 1,3-DCB, 20.41 μmole of 1,4-DCB and 20.57 μmole of 1,2-DCB).

The first DCB and MCB data points were taken on day 7. All the microcosms were run for 291 days. By day 40, dechlorination was observed in all soil microcosms except for the autoclaved controls (microcosms #8 and #9).

The DCB and MCB concentration profiles were similar to each other for the YE-fed microcosms #1 and #3, and exemplary results from microcosm #3 are presented in Figure 4.16. The MCB production in microcosm #3 accounted for 92% of the total (initial plus added) DCBs (but 96% of the total recovered chlorinated benzenes).

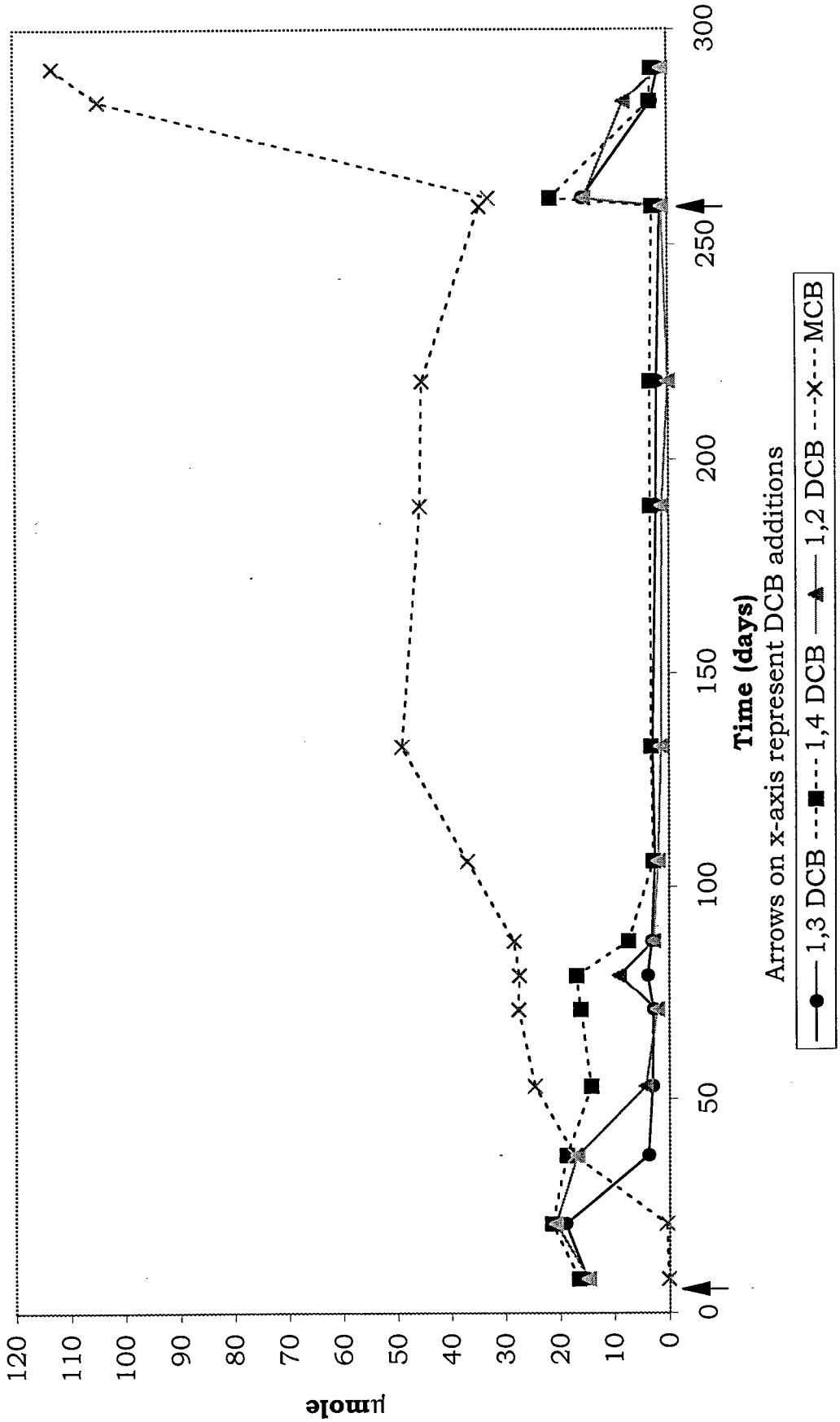


Figure 4.16 -- DCB and MCB Data for L Microcosm #3 (Fed)

The DCB and MCB concentration profiles for the YE-fed microcosms #2 and #4 were similar to each other and exemplary results from microcosm #4 are presented in Figure 4.17. The MCB production in microcosm #4 accounted for 66% of the total (added plus initial) DCBs (but 78% of the total recovered chlorinated benzenes).

The DCB and MCB concentration profiles for unfed microcosms #5, #6 and #7 were similar to each other and exemplary results from microcosm #5 are presented in Figure 4.18. The MCB production in microcosm #5 accounted for 76% of the total (initial plus added) DCBs (but 86% of the total recovered chlorinated benzenes).

The DCB and MCB concentration profiles for the autoclaved controls #8 and #9 were similar to each other and exemplary results from microcosm #8 are presented in Figure 4.19. The DCB and MCB concentration profiles #10, #11 and #12 were similar to each other and were similar to Figure 4.5.

On May 4, 1999 (day 221 for the source microcosms) three enrichments were prepared in basal medium. Enrichments #1 through #3 were made with a 1 ml, 2 ml and 5 ml mixed content inocula from microcosm #1. Enrichments #1 through #3 also received neat DCBs (2.3 µl of 1,2- and 1,3-DCB and 3 mg of 1,4-DCB) and 0.2 ml of YE stock. After day 50, it was evident that enrichments #1 and #2 did not have sufficient inocula and 4 ml of enrichment #1 and 3 ml of enrichment #2 were removed in the glovebox. Then 4 ml mixed contents of microcosm #3 was added to enrichment #1 and 3 ml mixed contents of microcosm #3 was added to enrichment #2.

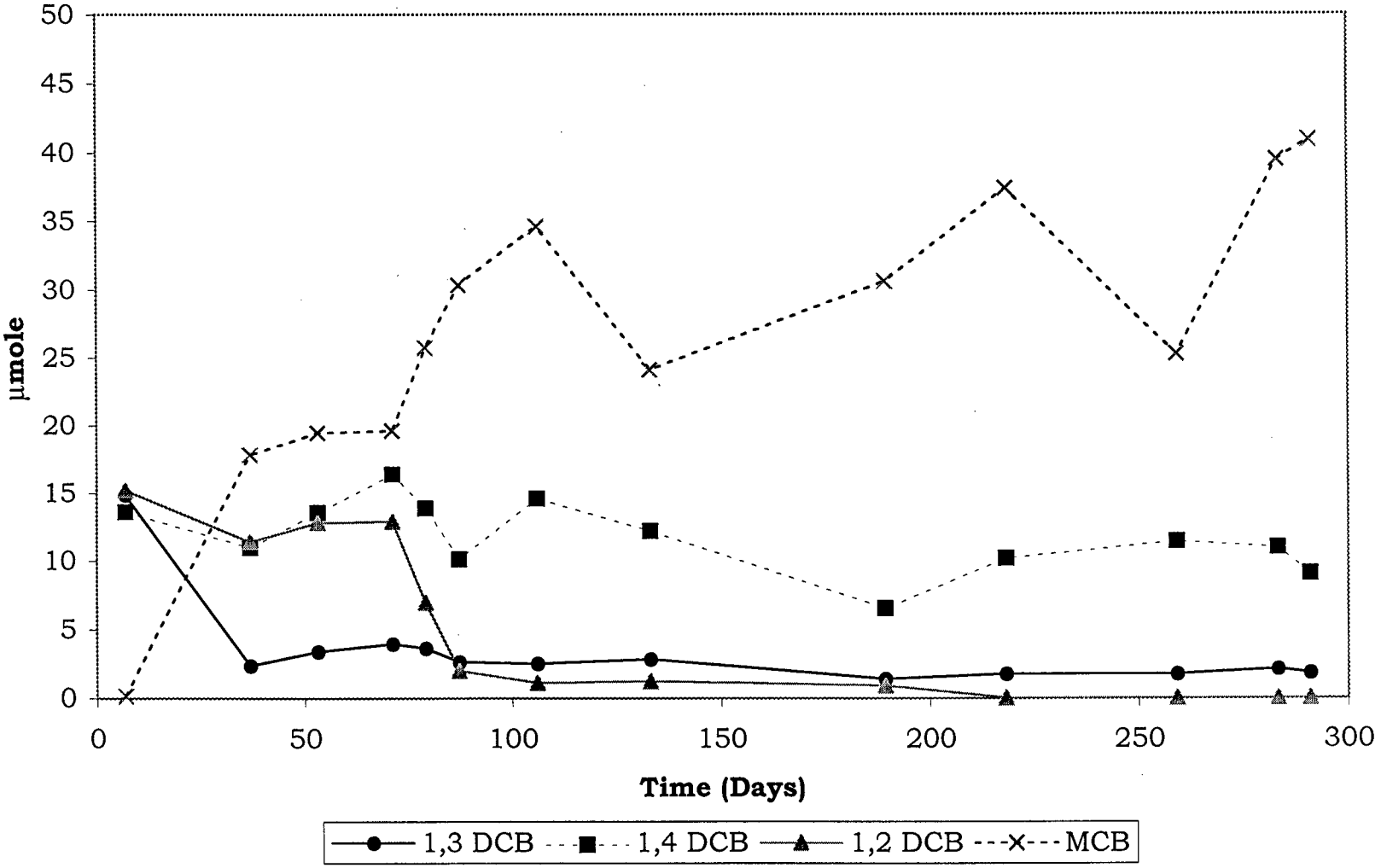


Figure 4.17 -- DCB and MCB Concentrations for L Microcosm # 4 (Fed)

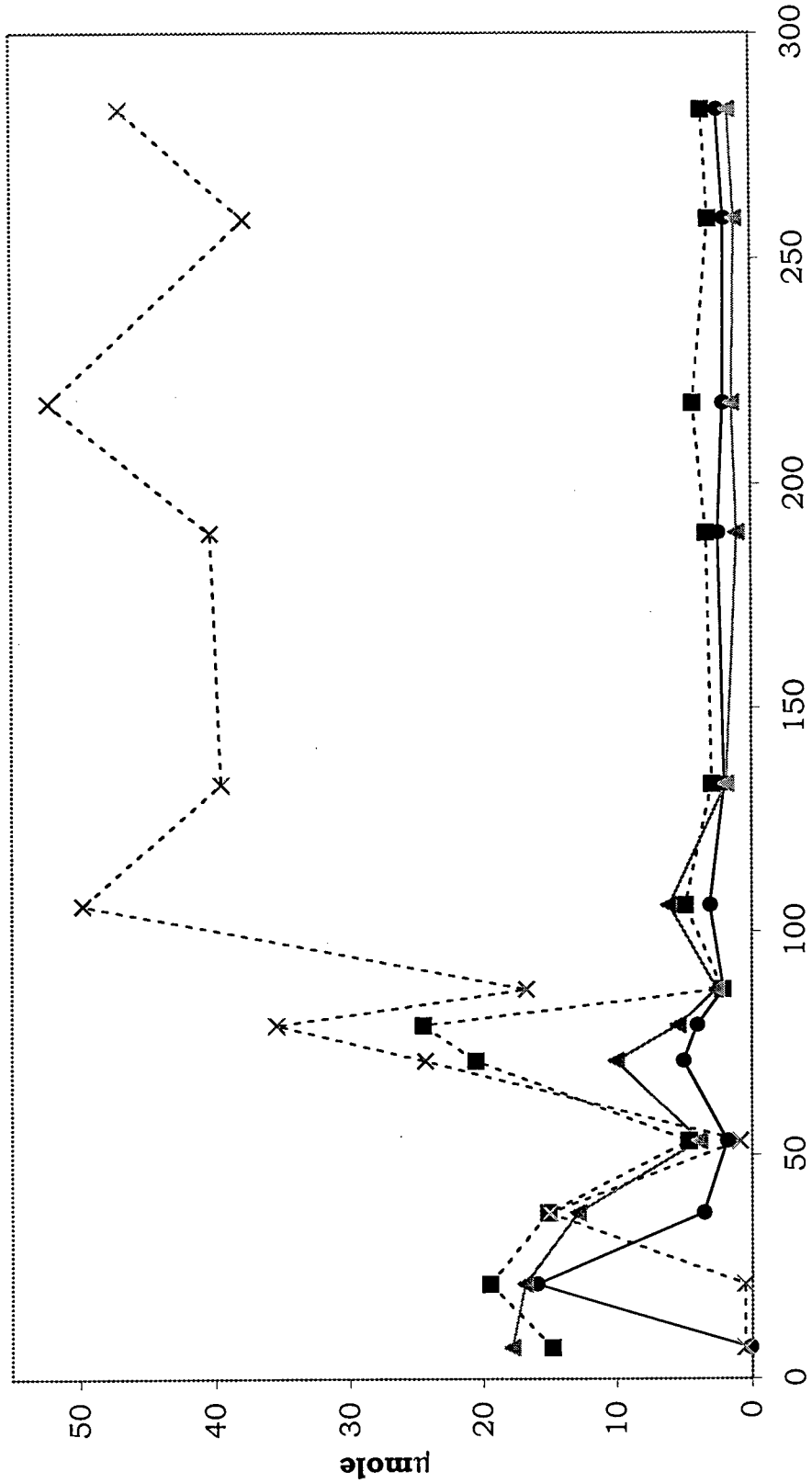


Figure 4.18 -- DCB and MCB Concentrations for L Microcosm #5 (Unfed)

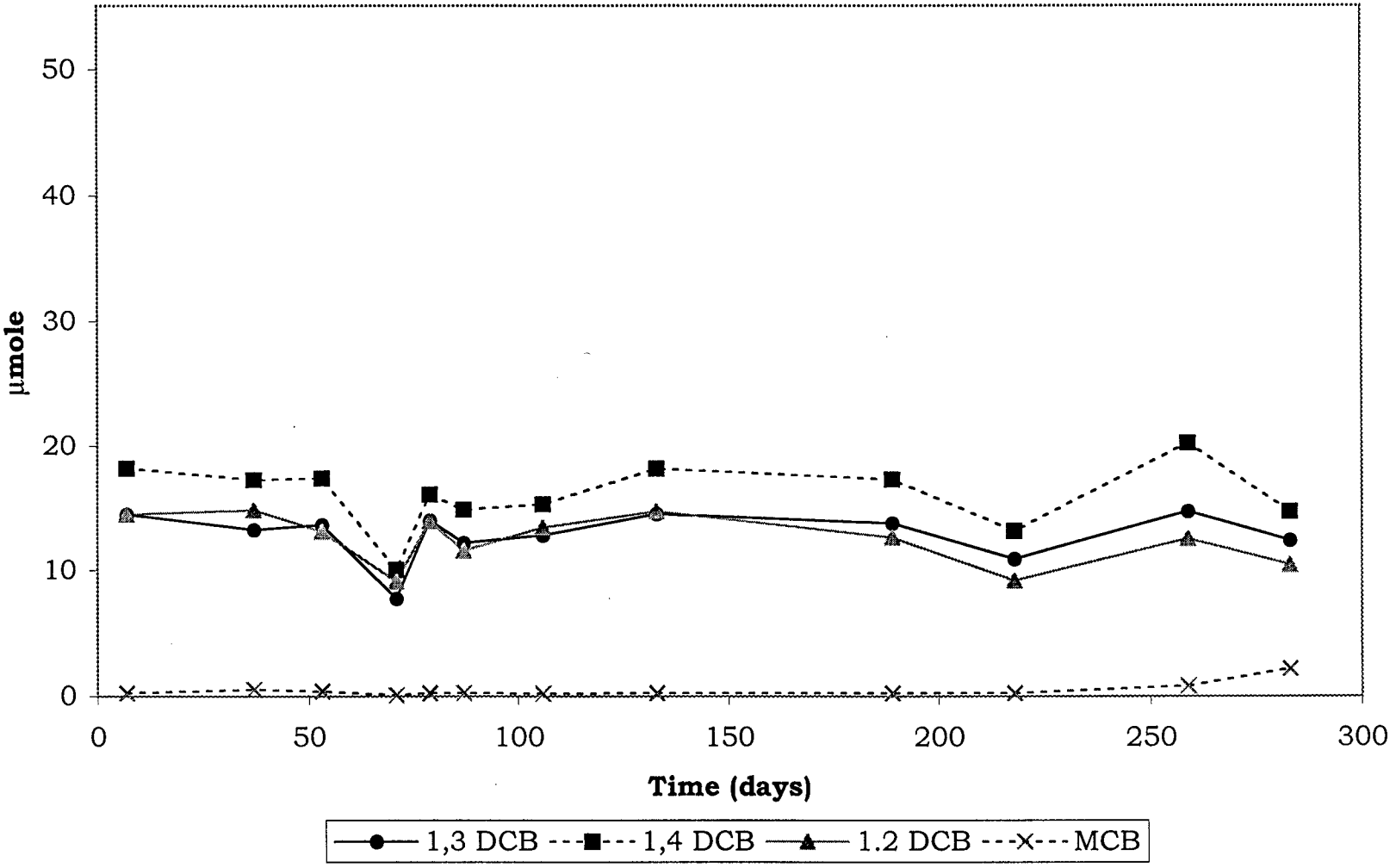


Figure 4.19 -- DCB and MCB Data for L Microcosm #8 (Autclaved Control)

Seven ml of autoclaved basal medium was then added back to microcosm #3.

The first DCB and MCB data points for the enrichments were taken on day 1. All the enrichments were run for 70 days. Dechlorination was only evident in enrichment #3. The DCB and MCB concentration profiles for enrichments #1 and #2 were similar to each other and are represented by enrichment #2 in Figure 4.20. The decrease in DCBs is probably due to losses through the septum. Figure 4.21 contains the DCB and MCB concentration profiles for L enrichment #3. The MCB production in enrichment #3 accounted for 32% of the total (added plus initial) DCBs (but 78% of the total recovered chlorinated benzenes).

For all active microcosms 1,3-DCB was the first DCB isomer degraded followed by 1,2-DCB and 1,4-DCB. For the one enrichment that had evidence of dechlorination, the order of dechlorination was 1,2-DCB followed by 1,3- and 1,4-DCB respectively.

4.G Robins Air Force Base at Well BIA4 at 25 Feet

The R-1-d microcosms were prepared on December 2, 1998. Table 4.11 contains the sample data.

Table 4.11 -- R-1-d Sample Data

Moisture Content (% of wet weight)	12.39
Groundwater Alkalinity (meq/L)	9.1
Groundwater + Soil Alkalinity (meq/L)*	9.25
Conductivity (mS/cm)	937
Groundwater + Soil pH*	6.67

* At soil/water ratio used in microcosms

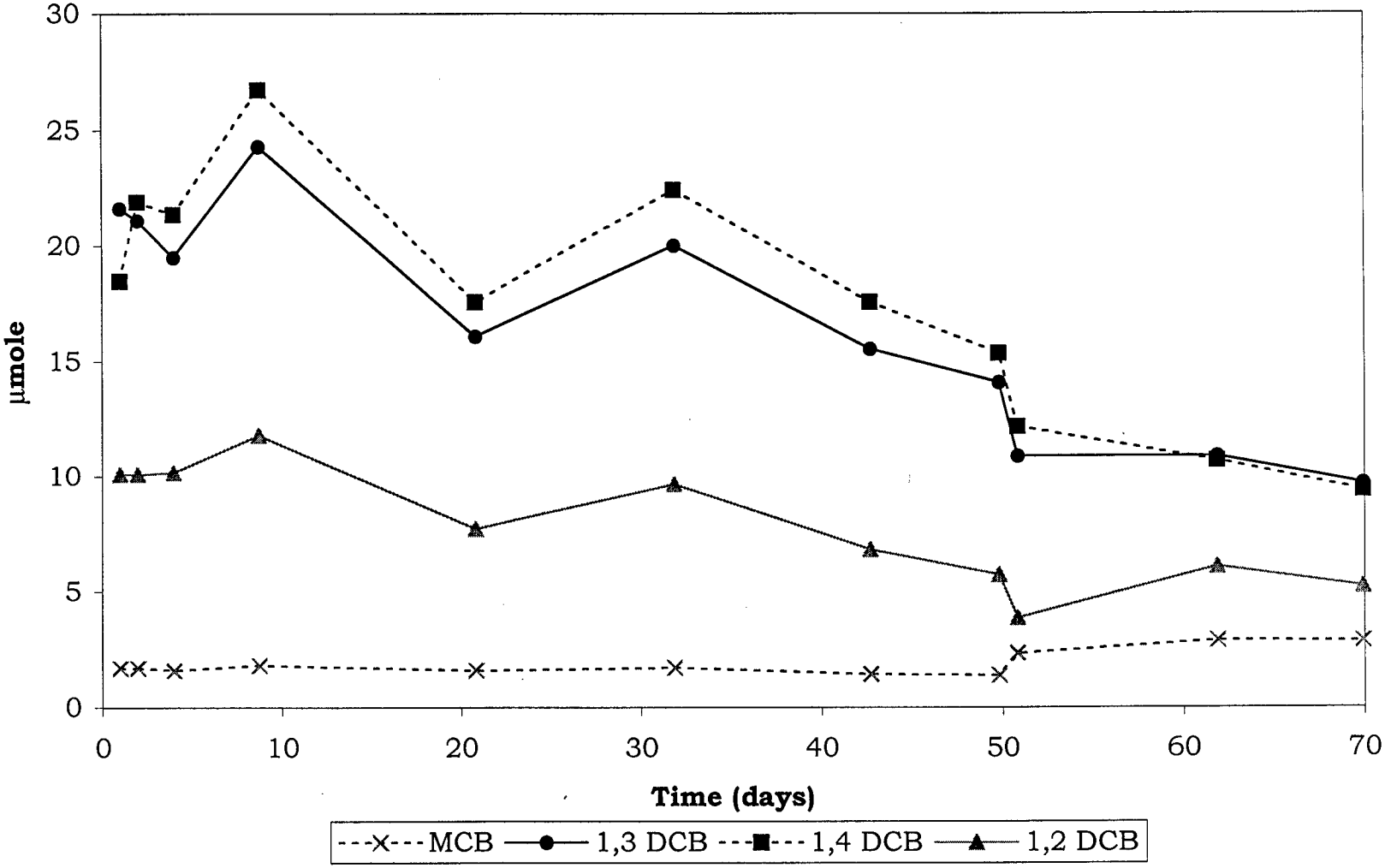


Figure 4.20 -- DCB and MCB Concentrations for L Enrichment #2

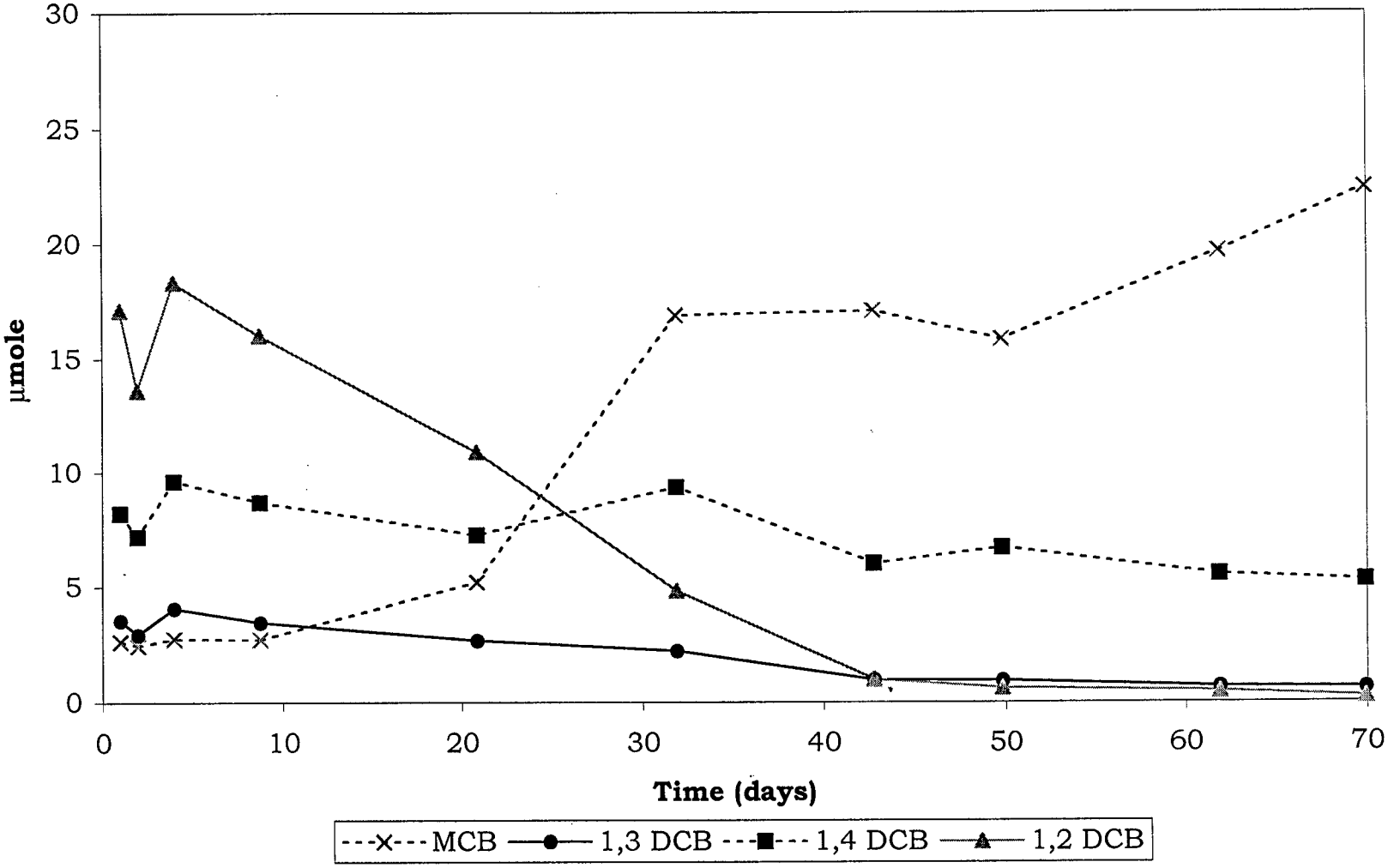


Figure 4.21 -- DCB and MCB Concentrations for L Enrichment #3

Fifteen microcosms were prepared for this site. Three were used for calibration purposes, and from the calibration the background concentrations of DCBs at the site were determined (Table 4.12). The remaining 12 microcosms were monitored

Table 4.12 -- R-1-d Background Data

	Background (μmole)
MCB	0.22
1,3-DCB	0.08
1,4-DCB	0.11
1,2-DCB	0.18

throughout the experiment. Microcosms #1 through #9 were made with 50 g of soil and 92 g of groundwater and DCBs (8 g of the DCB stock 3.G.1a). Once dechlorination was observed, DCBs were again added. After sufficient data had been gathered for the natural-attenuation study, high levels of DCBs were added to several microcosms. The total levels of DCBs in the microcosms were the sums of the background and the added levels (Tables 4.12, 4.13, 4.14).

Table 4.13 -- DCB Additions to R-1-d Microcosms #5-#12
Additions Expressed in μmoles

Microcosms		day 0	day 45	Total
#5-#7	1,3-DCB	0.87	0.87	1.74
	1,4-DCB	0.85	0.85	1.70
	1,2-DCB	1.18	1.18	2.36
#8-#12	1,3-DCB	0.87		0.87
	1,4-DCB	0.85		0.85
	1,2-DCB	1.18		1.18

Table 4.14 -- DCB Additions to R-1-d Microcosms #1 - #4

Microcosm		day 0	day 45	day 55	day 75	day 87	day 134	day 174	Total
#1	1,3-DCB	0.87	0.87	20.29		20.29	20.29	20.29	82.90
	1,4-DCB	0.85	0.85	21.77			19.05	20.41	62.93
	1,2-DCB	1.18	1.18	20.57		20.57	20.57	20.57	84.64
#2	1,3-DCB	0.87	0.87	20.29					22.03
	1,4-DCB	0.85	0.85	19.05					20.75
	1,2-DCB	1.18	1.18	20.57					22.93
#3	1,3-DCB	0.87	0.87	0.87	0.87			20.29	23.77
	1,4-DCB	0.85	0.85	0.85	0.85			21.77	25.17
	1,2-DCB	1.18	1.18	1.18	1.18			20.57	25.29
#4	1,3-DCB	0.87	0.87	0.87	0.87	0.87		20.29	24.64
	1,4-DCB	0.85	0.85	0.85	0.85	0.85		21.09	25.34
	1,2-DCB	1.18	1.18	1.18	1.18	1.18		20.57	26.47

In addition, microcosms #1 through #4 received 100 mg/L YE (0.2 ml of the YE stock 3.G.1c). Once VFA data confirmed that the electron donor had been consumed, more YE was added. Table 4.15 contains the YE addition data. Microcosms #1 and #2 received an

Table 4.15 -- YE Additions to R-1-d Microcosms

Microcosm	day 0	day 56	day 76	day 88	day 131	day 135	day 174	day 175
#1	X	X		X		X		X
#2	X	X			X		X	
#3	X		X					X
#4	X		X					X

addition of $NaHCO_3$ (bringing the total alkalinity up to 50 meq/L) on day 55 and microcosms #3 and #4 received an addition of $NaHCO_3$ day 174. Microcosms #8 and #9 were autoclaved. Microcosms #10 through #12 were made with 100 ml of distilled water, DCBs (8 g of the DCB stock) and were autoclaved (Table 4.13).

The first DCB and MCB data points were taken on day 1. All the microcosms were run for 219 days. Dechlorination was evident after 18 days. Figure 4.22 contains the DCB and MCB concentration profiles for microcosm #1 (fed) and Figure 4.23 contains the equivalents profile for microcosm #1. The MCB production in microcosm #1 accounted for 74% of the total (initial plus added) DCBs (the MCB production was the sum of the MCB level before the purge and after the purge subtracted from the initial MCB concentration). The MCB production accounted for 99% of the total recovered chlorinated benzenes on day 174.

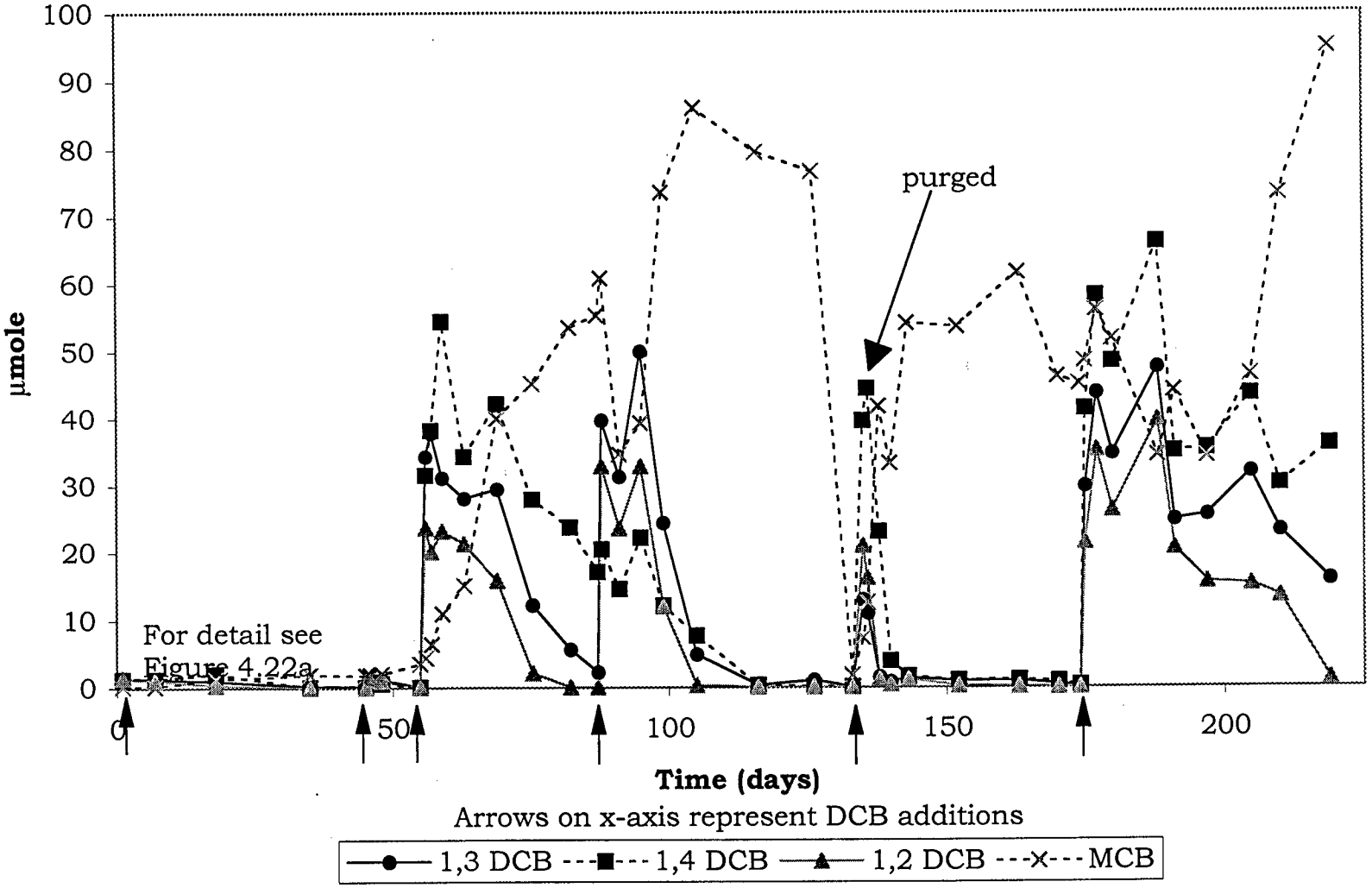


Figure 4.22 -- DCB and MCB Concentrations for R-1-d Microcosm #1 (Fed)

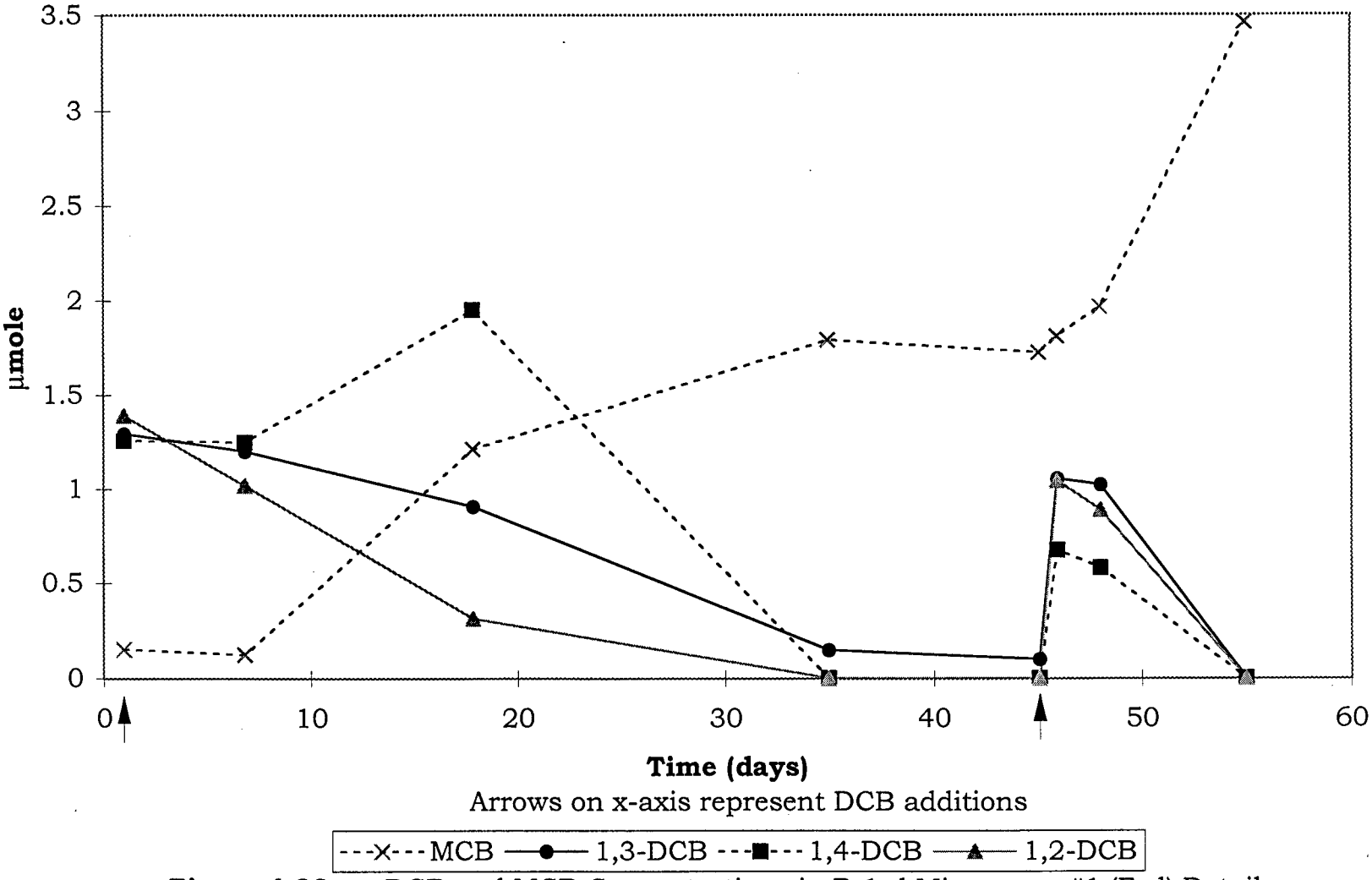
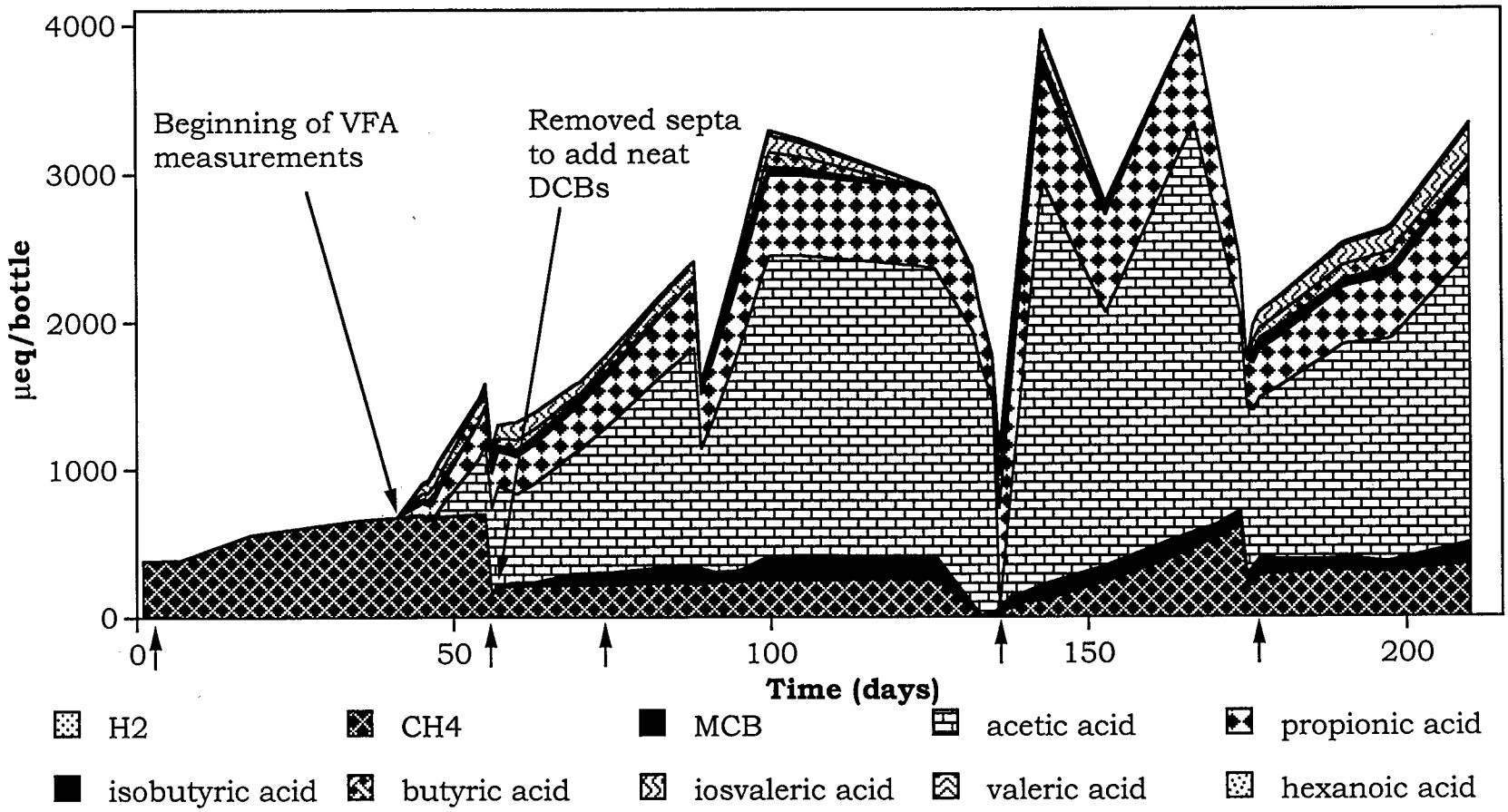


Figure 4.22a -- DCB and MCB Concentrations in R-1-d Microcosm #1 (Fed) Detail



Arrows on x-axis represent YE additions

Figure 4.23 -- Equivalents Profile for R-1-d Microcosm #1 (Fed)

Figure 4.24 contains the DCB and MCB concentration profiles for microcosm #2 (fed) and Figure 4.25 contains the equivalents profile for microcosm #2. The MCB production in microcosm #2 accounted for 98% of the total (initial plus added) DCBs (but 98% of the total recovered chlorinated benzenes).

The DCB and MCB concentration profiles were similar among microcosms #5, #6, and #7 and are exemplified by Figure 4.26. The MCB production accounted for 43% of the total (initial plus added) DCBs in microcosm #7 (but 100% of the total recovered chlorinated benzenes).

The DCB and MCB concentration profiles were similar among autoclaved microcosms # 8 and #9 and are exemplified by Figure 4.27.

On day 100, a peak that eluted at the same time as benzene in microcosms #1 and #2, began to noticeably increase. Evidence to support the hypothesis that the peak was benzene was gathered by identifying the peak as benzene on a GC/ mass spectrometer (MS) (Hewlett Packard series 5890 GC and a Hewlett Packard series 5971 MS). The film was HP-5 crosslinked 5% PH Me Siloxane. The column was 30 m long with an inside diameter of 0.25 mm, a film thickness of 0.25 μm and a phase ratio of 250. A 0.5-ml headspace sample from microcosm #1 was injected into the GC/MS (Figure 4.28). In addition a 0.5-ml headspace injection was made from a benzene standard (Figure 4.29). The hypothetical benzene peak from microcosm #1 eluted at the same time on this column as the peak from the benzene standard (2.6 min). Thus, there was co-

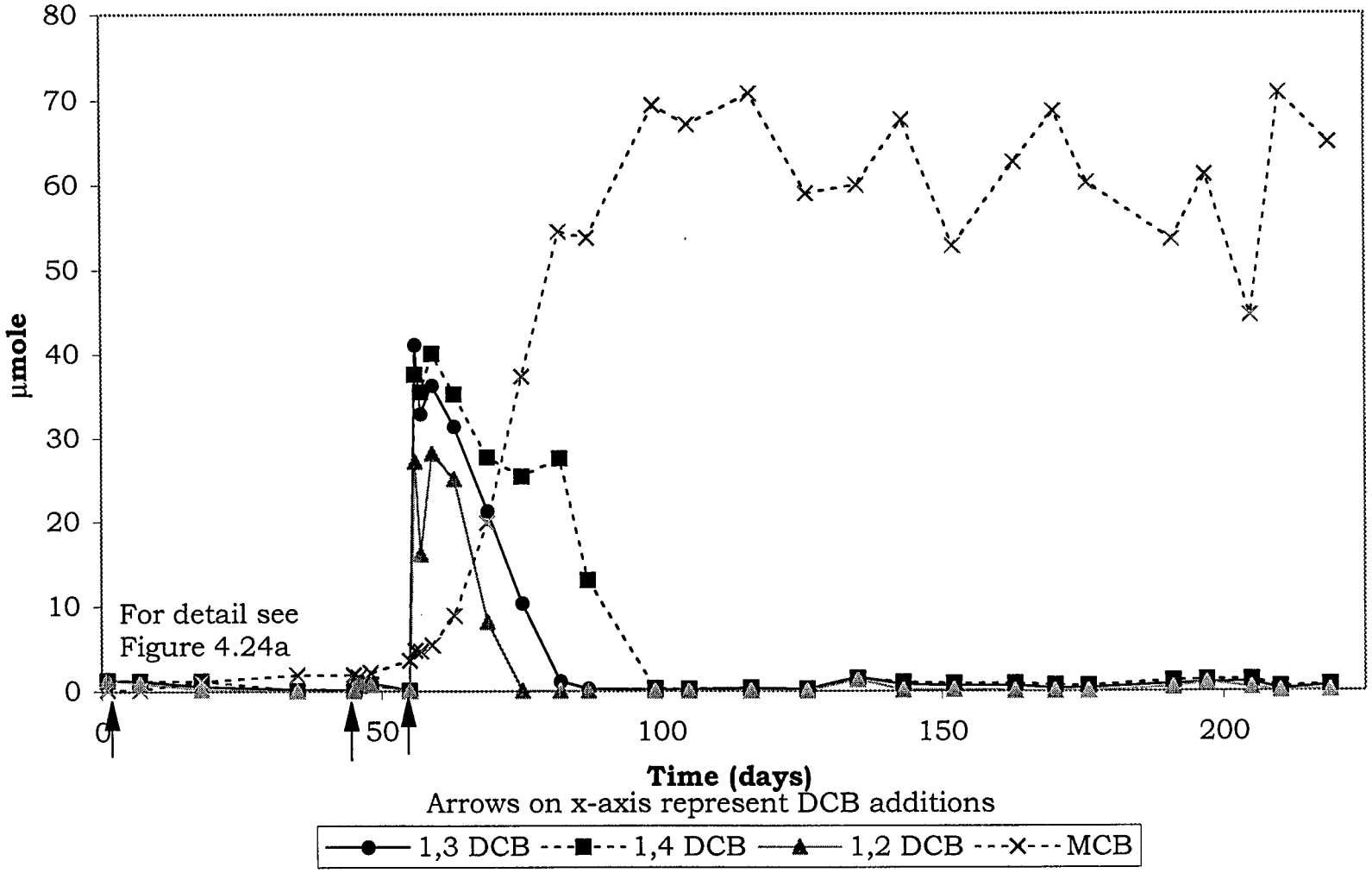


Figure 4.24 -- DCB and MCB Concentrations for R-1-d Microcosm #2 (Fed)

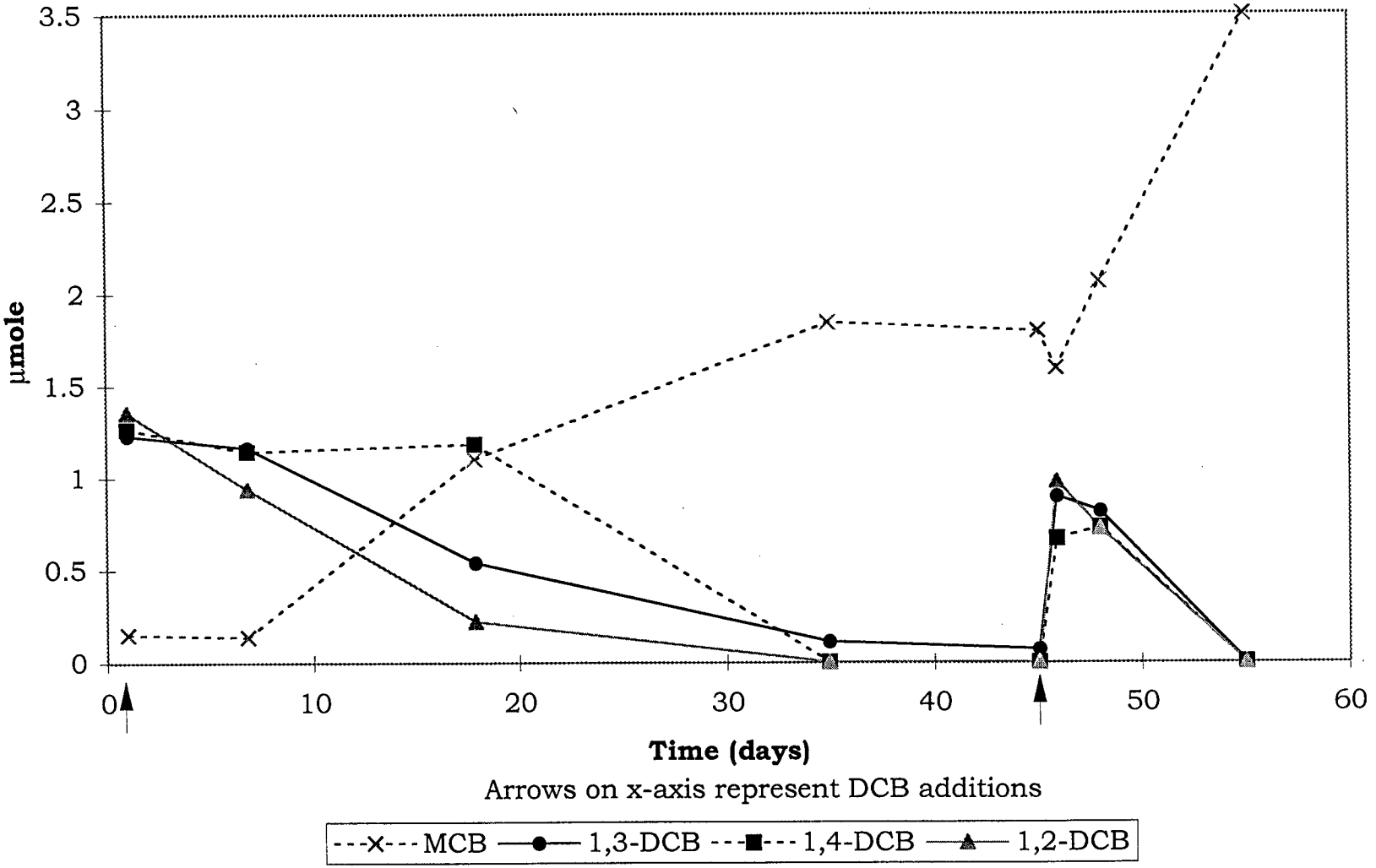


Figure 4.24a -- DCB and MCB Concentrations for R-1-d Microcosm #2 (Fed) Detail

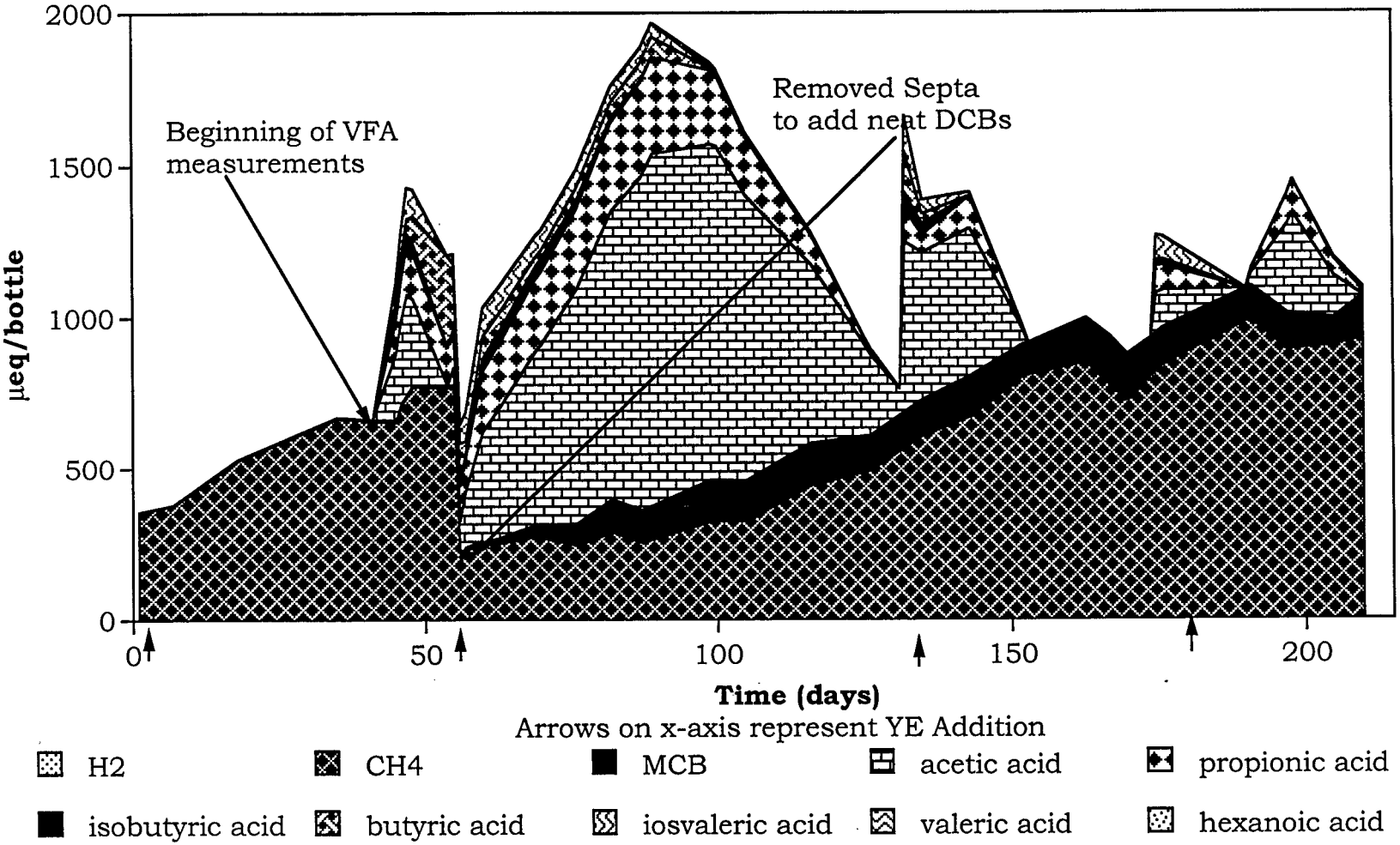


Figure 4.25 -- Eqivalents Profile for R-1-d Microcosm #2 (Fed)

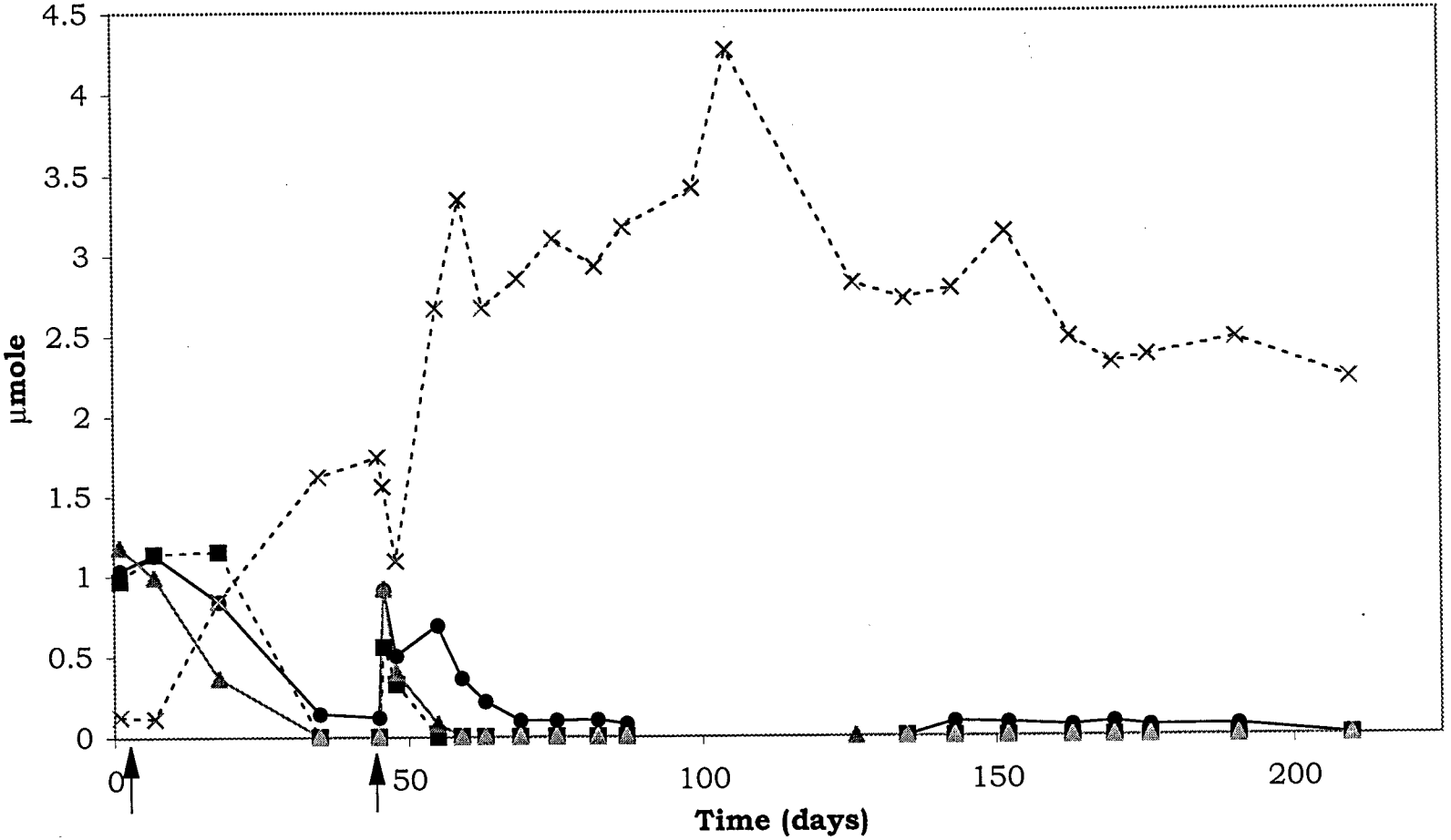


Figure 4.26 -- DCB and MCB Concentrations for R-1-d Microcosm #7 (Unfed)

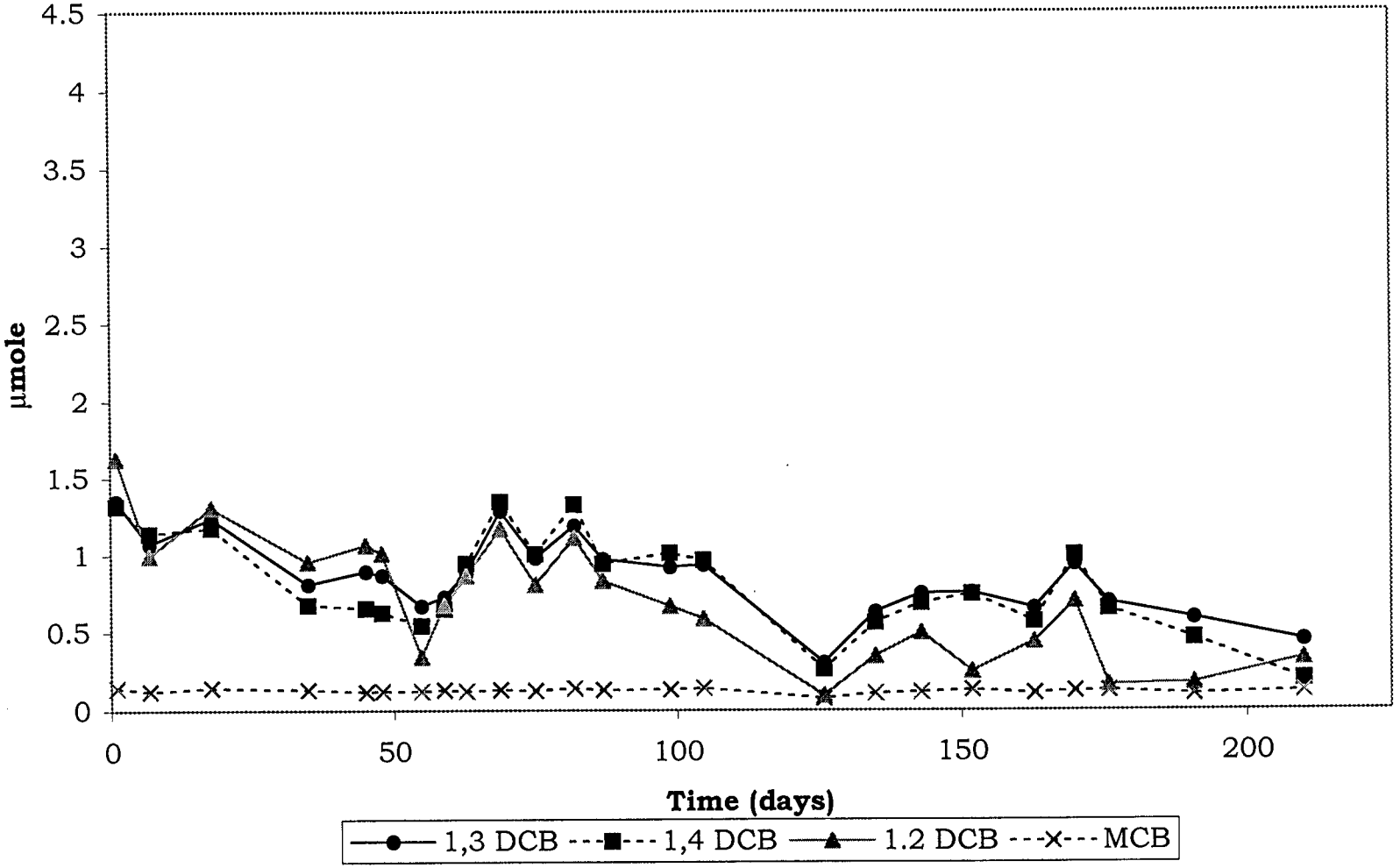


Figure 4.27 -- DCB and MCB Concentrations for R-1-d Microcosm #8 (Autoclaved Control)

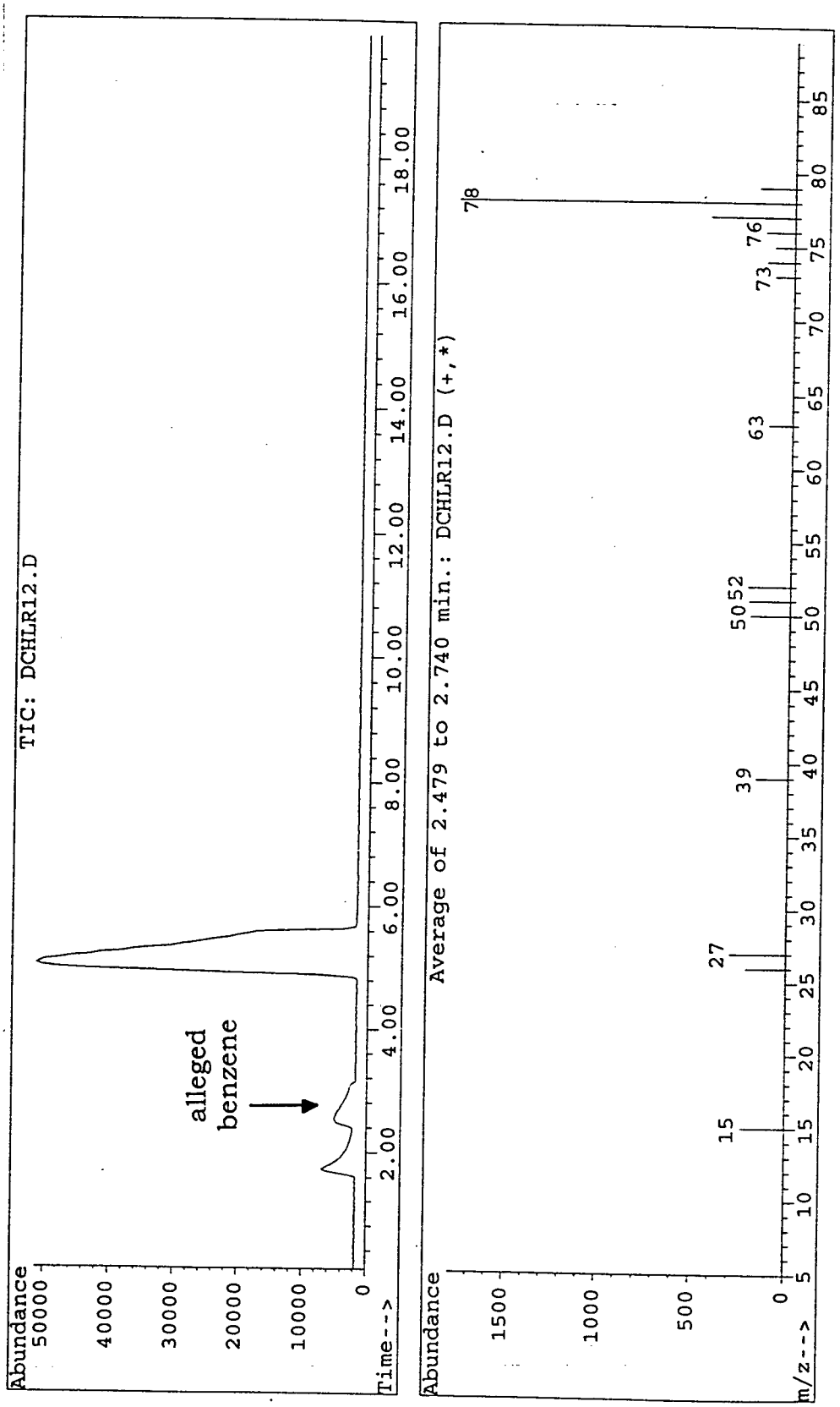


Figure 4.28 -- GC/MS Plot for R-1-d Microcosm #1

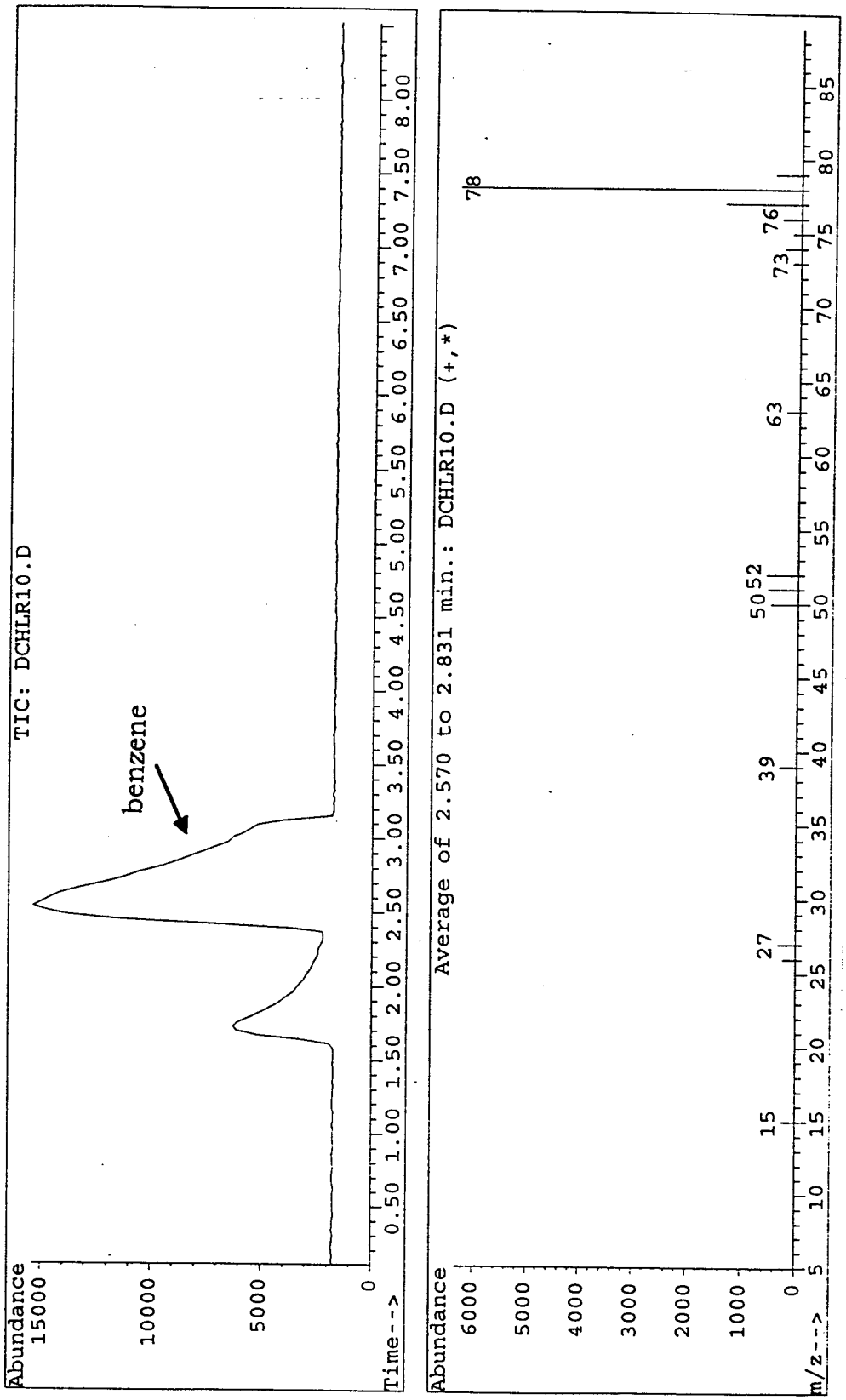


Figure 4.29 -- GC/MS Plot for Benzene Standard

evolution of the unknown and authentic benzene on two completely different columns. Finally a 0.5-ml injection of air was run as a negative control and as expected, no benzene peak eluted (Figure 4.30). In addition, the MS of the hypothesized benzene peak from microcosm #1 was matched as benzene (90% confidence) from the data base in the GC/MS (Figure 4.31). The GC/MS was run in the SIM mode where only certain fragments (m/z) were plotted. The fragments plotted were: 37, 39, 50, 51, 52, 55, 73, 74, 75, 76, 77, 78, 79, 111, 112, 113, 114, 146, 147, 148 and 150. These fragments were chosen because they were the key fragments for benzene, MCB, 1,3-, 1,4- and 1,2-DCB. The benzene concentration profiles for microcosms #1 and #2 are displayed in Figures 4.32 and 4.33. The total benzene accumulation accounted for about 1% of the total recovered MCB in both microcosms #1 and #2 (the benzene accumulation in microcosm #1 was the sum of the benzene level before the purge and after the purge).

On January 16, 1999 four enrichments were made in 94 ml of basal media. Enrichments #1 through #4 were each made with and 8 ml inoculum of microcosms #1 through #4, respectively. Enrichments #1 through #4 also received 8 ml of DCB stock and 0.2 ml of YE stock. In addition, enrichments #2 and #4 received another 8-ml inoculum from microcosms #3 and #4 ten days later. After the DCBs and YE had been consumed, more DCBs and YE were added. Table 4.16 and 4.17 detail the DCB and YE additions.

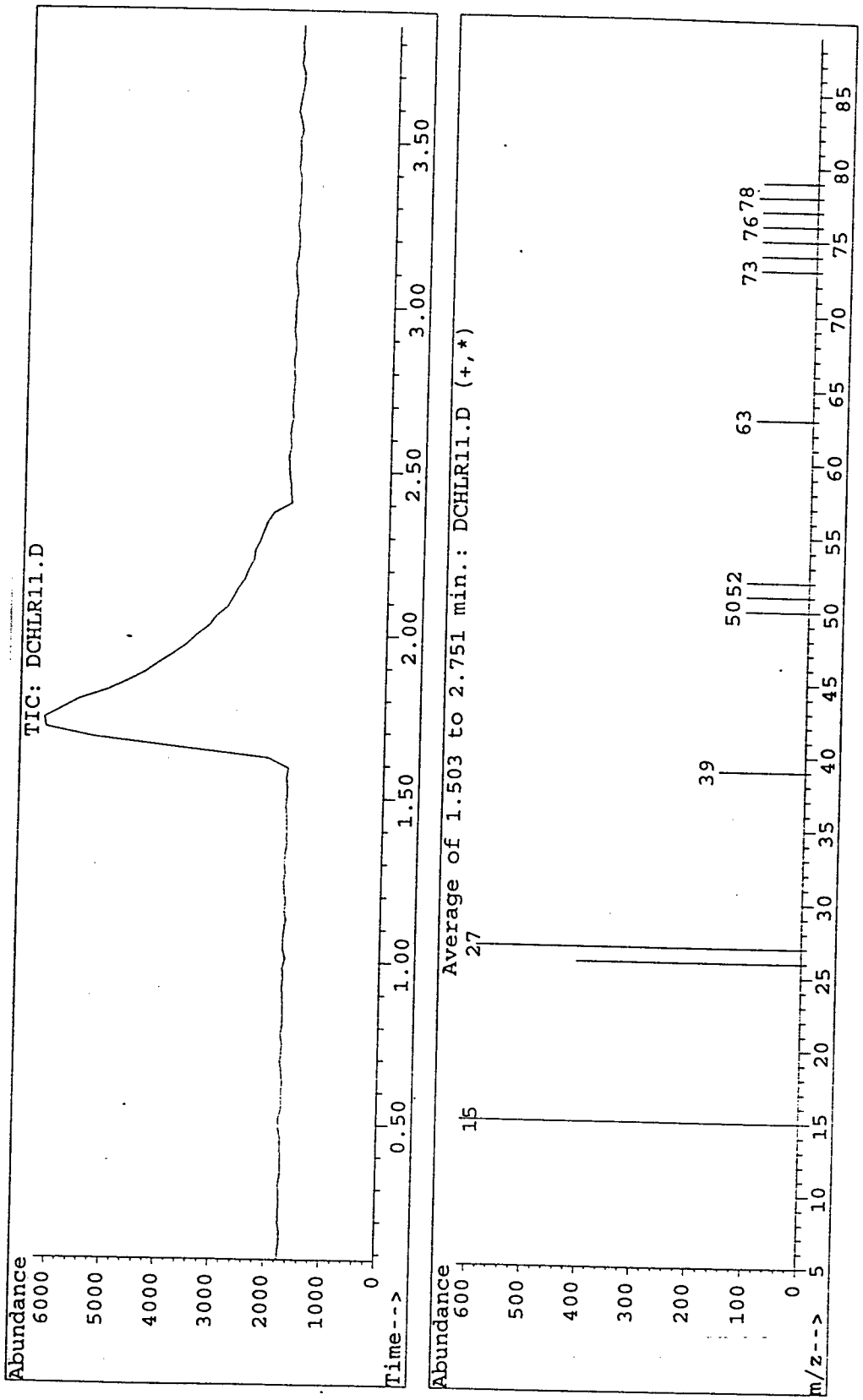


Figure 4.30 -- GC/MS Plot for the Negative Control

Libr Searched : C:\DATABASE\NBS75K.L
Quality : 91
ID : Benzene

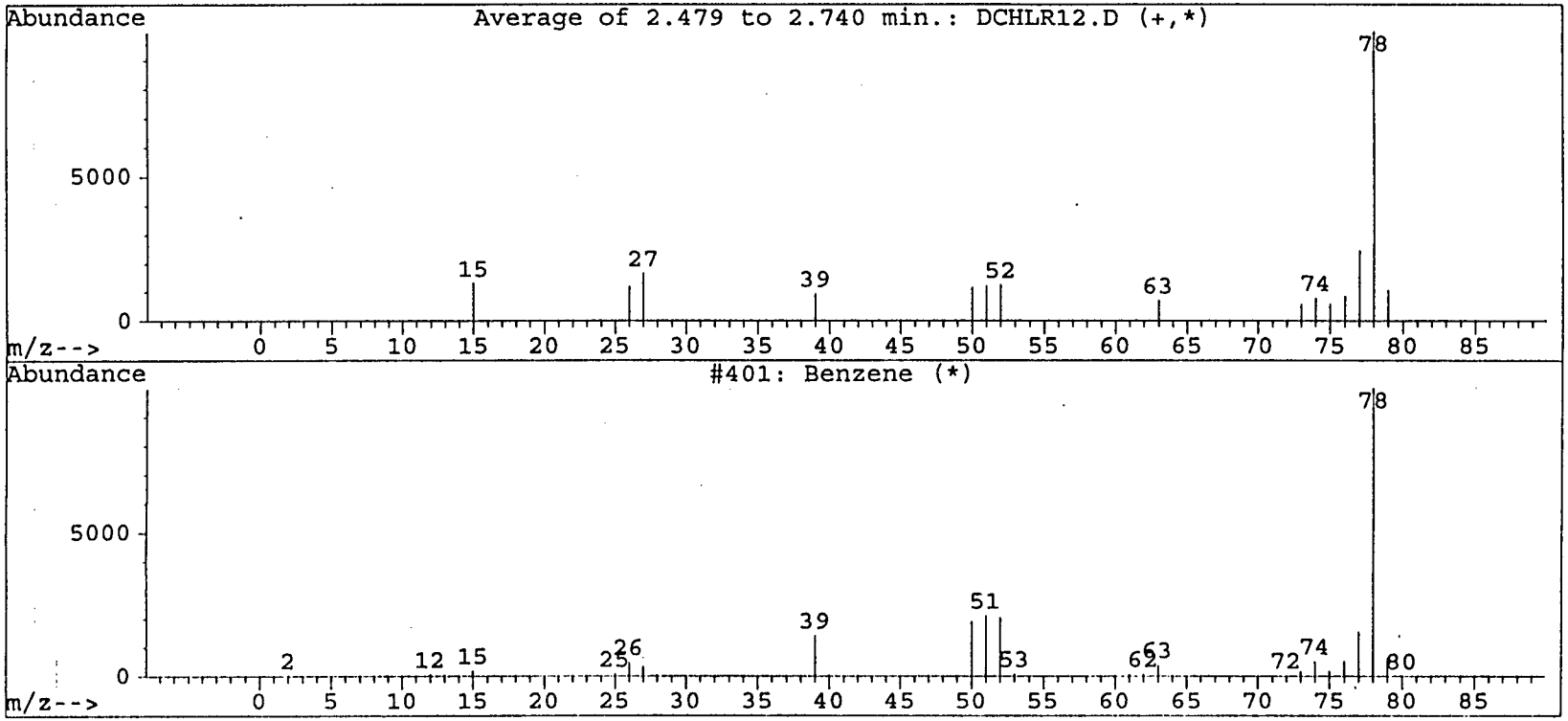


Figure 4.31 -- GC/MS Identification of the Peak at 2.6 min as Benzene

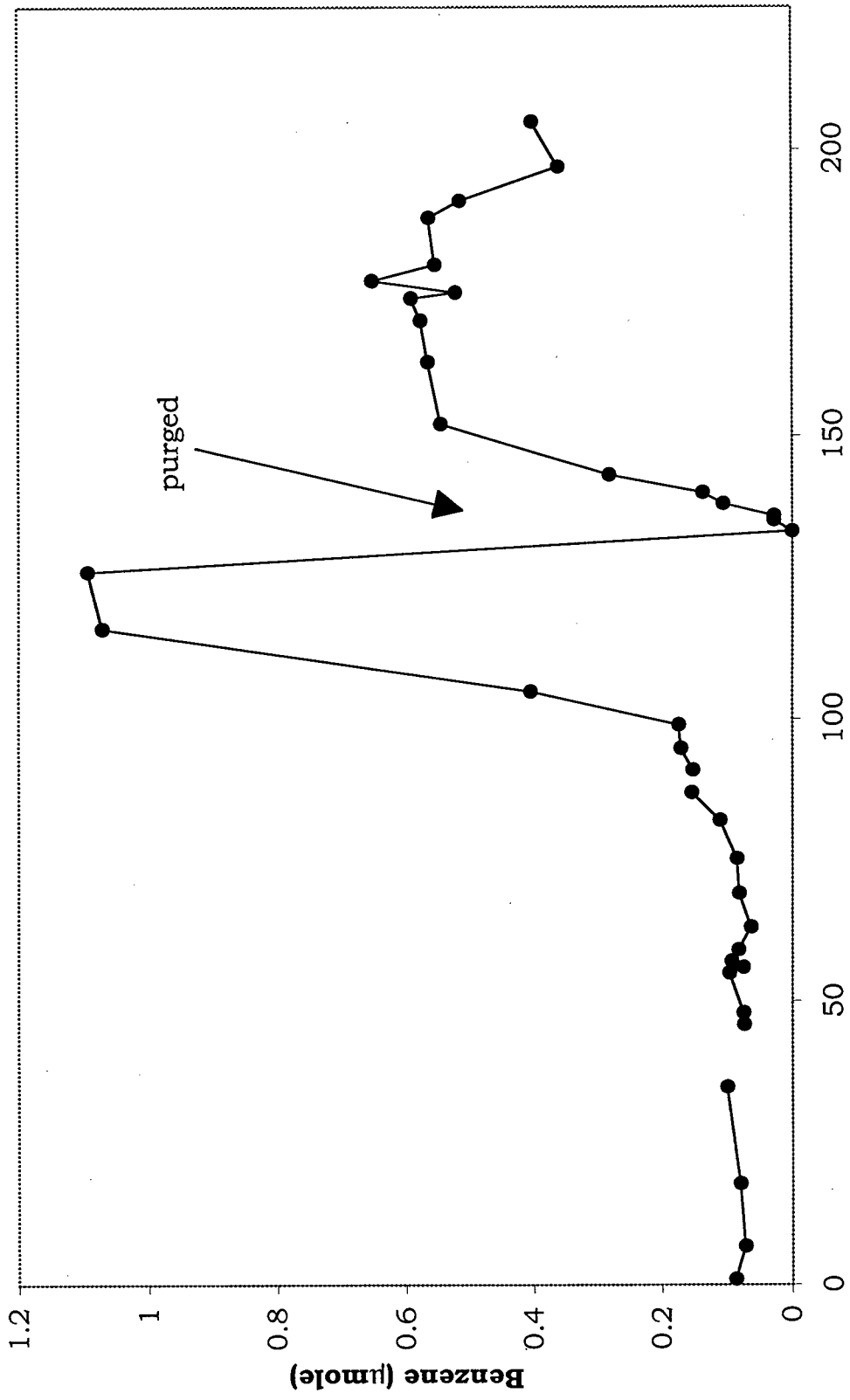


Figure 4.32 -- Benzene Concentration for R-1-d Microcosm #1 (Fed)

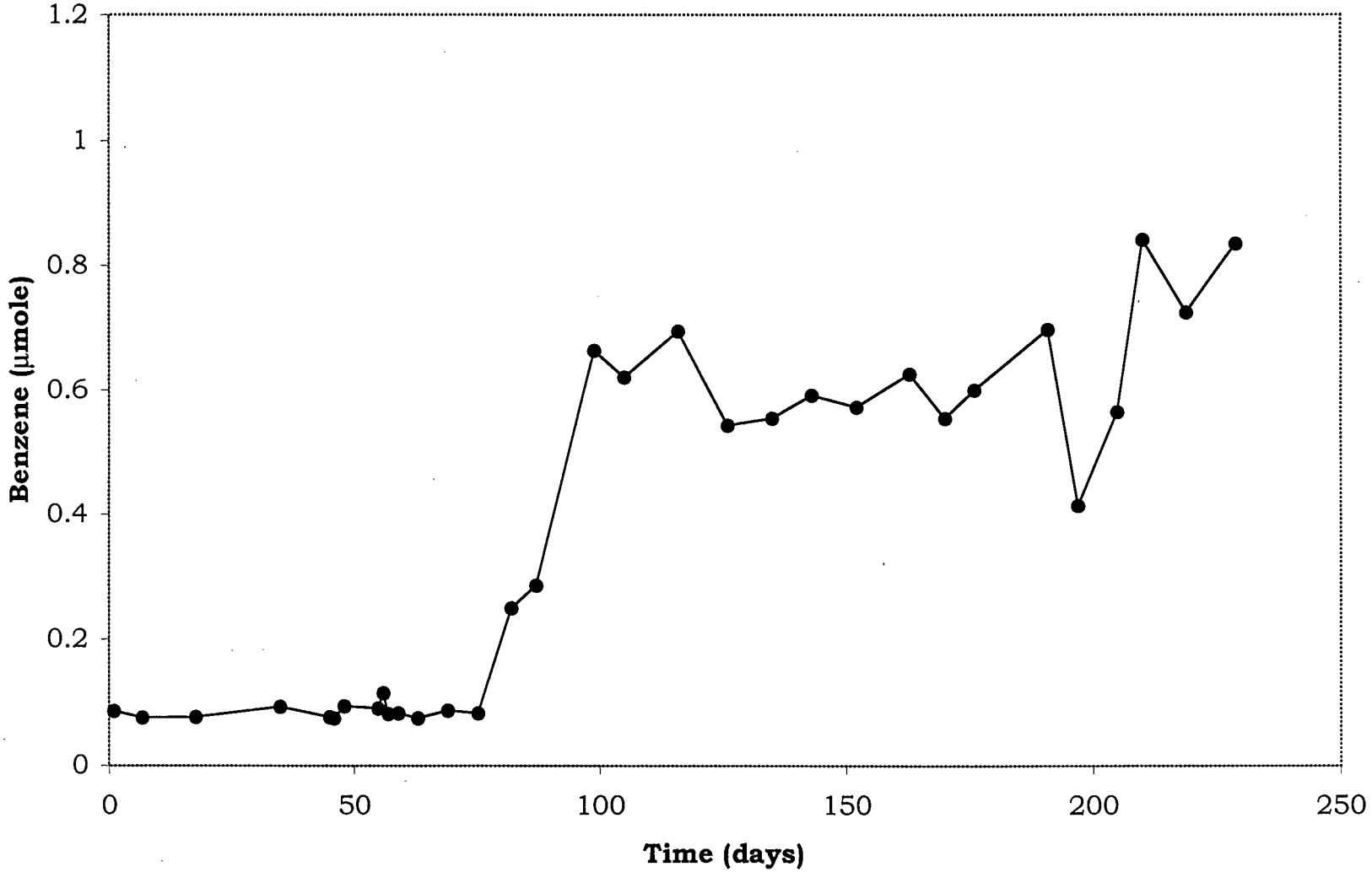


Figure 4.33 -- Benzene Concentration for R-1-d Microcosm #2 (Fed)

Table 4.16 -- DCB Additions to R-1-d Enrichments

Enrichments		day 0	day 42	day 98	day 129	day 149	day 152	Total
#1	1,3-DCB	0.87		0.87			20.29	22.03
	1,4-DCB	0.85		0.85			22.46	24.16
	1,2-DCB	1.18		1.18			20.57	22.93
#2	1,3-DCB	0.87		0.87	0.87		20.29	22.9
	1,4-DCB	0.85		0.85	0.85		23.14	25.69
	1,2-DCB	1.18		1.18	1.18		20.57	24.11
#3	1,3-DCB	0.87	0.87		20.29			22.03
	1,4-DCB	0.85	0.85					1.7
	1,2-DCB	1.18	1.18		20.57			22.93
#4	1,3-DCB	0.87	0.87			20.29		22.03
	1,4-DCB	0.85	0.85			23.82		25.53
	1,2-DCB	1.18	1.18			20.57		22.93

Table 4.17 -- YE Additions to R-1-d Enrichments

Enrichment	day 0	day 10	day 43	day 98	day 129	day 130	day 153
1	X			X	X		X
2	X	X		X		X	X
3	X	X	X			X	
4	X		X		X		X

All the enrichments were run for 174 days. Dechlorination was evident after 18 days in enrichments #3 and #4, after 30 days in enrichment #1 and after 80 days in enrichment #2. The DCB and MCB profiles and equivalents profiles were similar for enrichments #1 and #2 and are represented by enrichment #2 in Figures 4.34 and Figure 4.35. The MCB production in enrichment #2 accounted for 0.7% of the total (added plus initial) DCBs (but 3% of the total recovered chlorinated benzenes).

The DCB and MCB level profiles and equivalents profiles were similar for enrichments #3 and #4 and are represented by enrichment #4 in Figure 4.36 and 4.37. The MCB production in enrichment #4 accounted for 25% of the total (initial plus added) DCBs (but 21% of the total recovered chlorinated benzenes).

4.H Robins Air Force Base Well BIA4 at 17 feet

The R-1-s microcosms were prepared on November 24, 1998. Table 4.18 contains the sample data.

Fifteen microcosms were prepared for this site. Three were used for calibration purposes, and from the calibration the background concentrations of DCBs and MCB at the site were determined (Table 4.19). The remaining 12 microcosms were

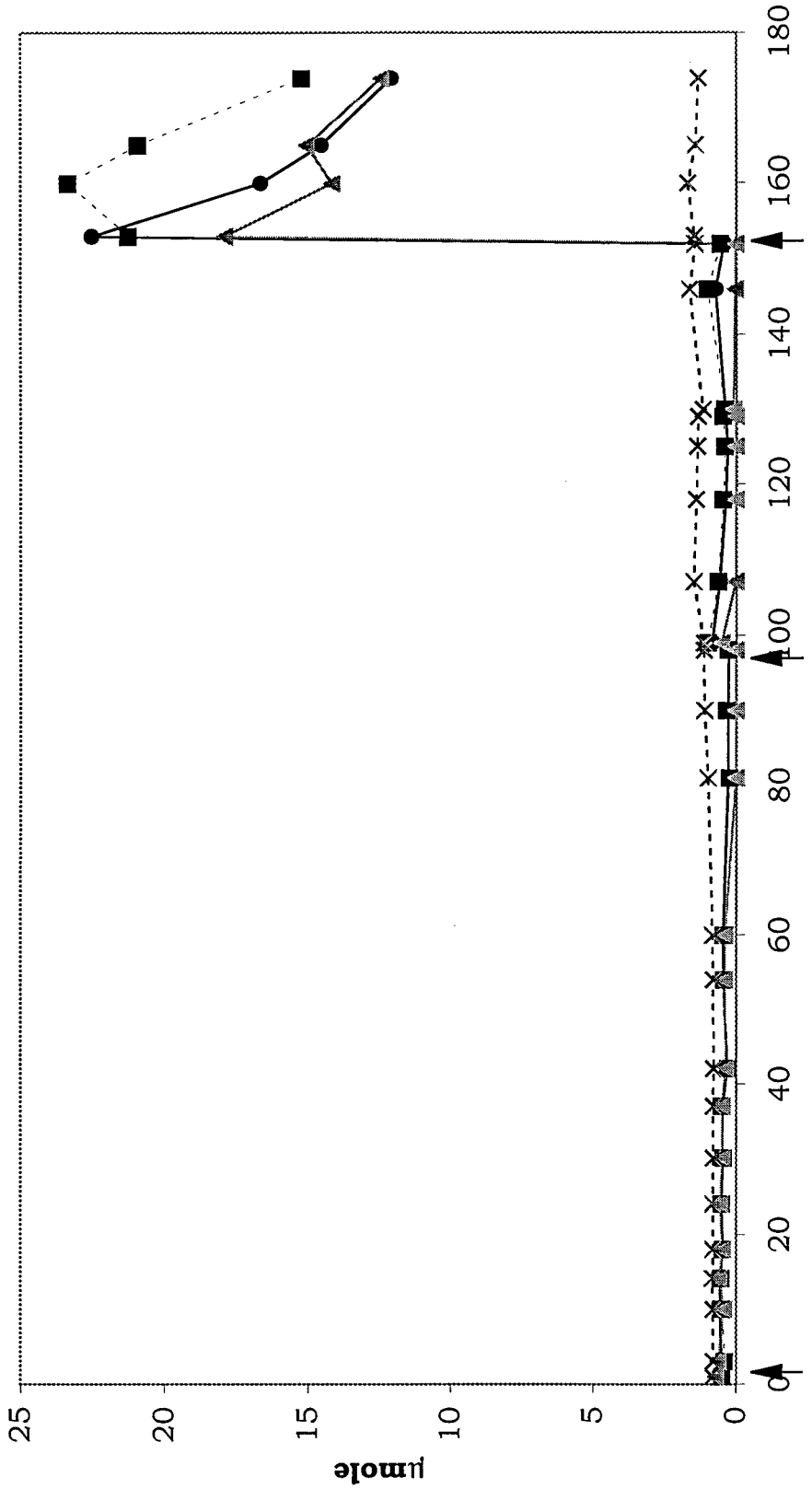
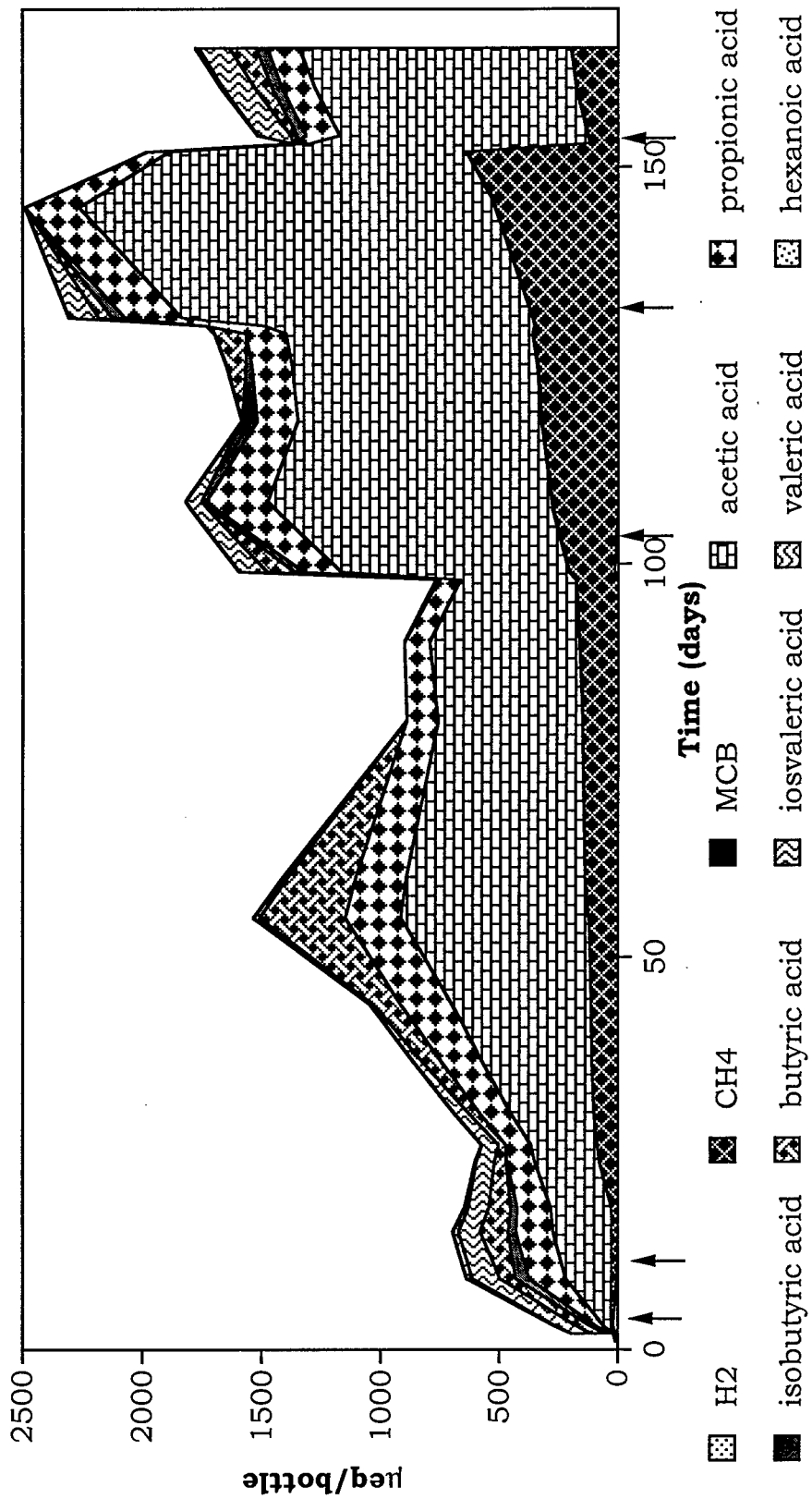


Figure 4.34 -- DCB and MCB Data for R-1-d Enrichment #2



Arrows on x-axis represent YE additions

Figure 4.35 -- Equivalents Profile for R-1-d Enrichment #2

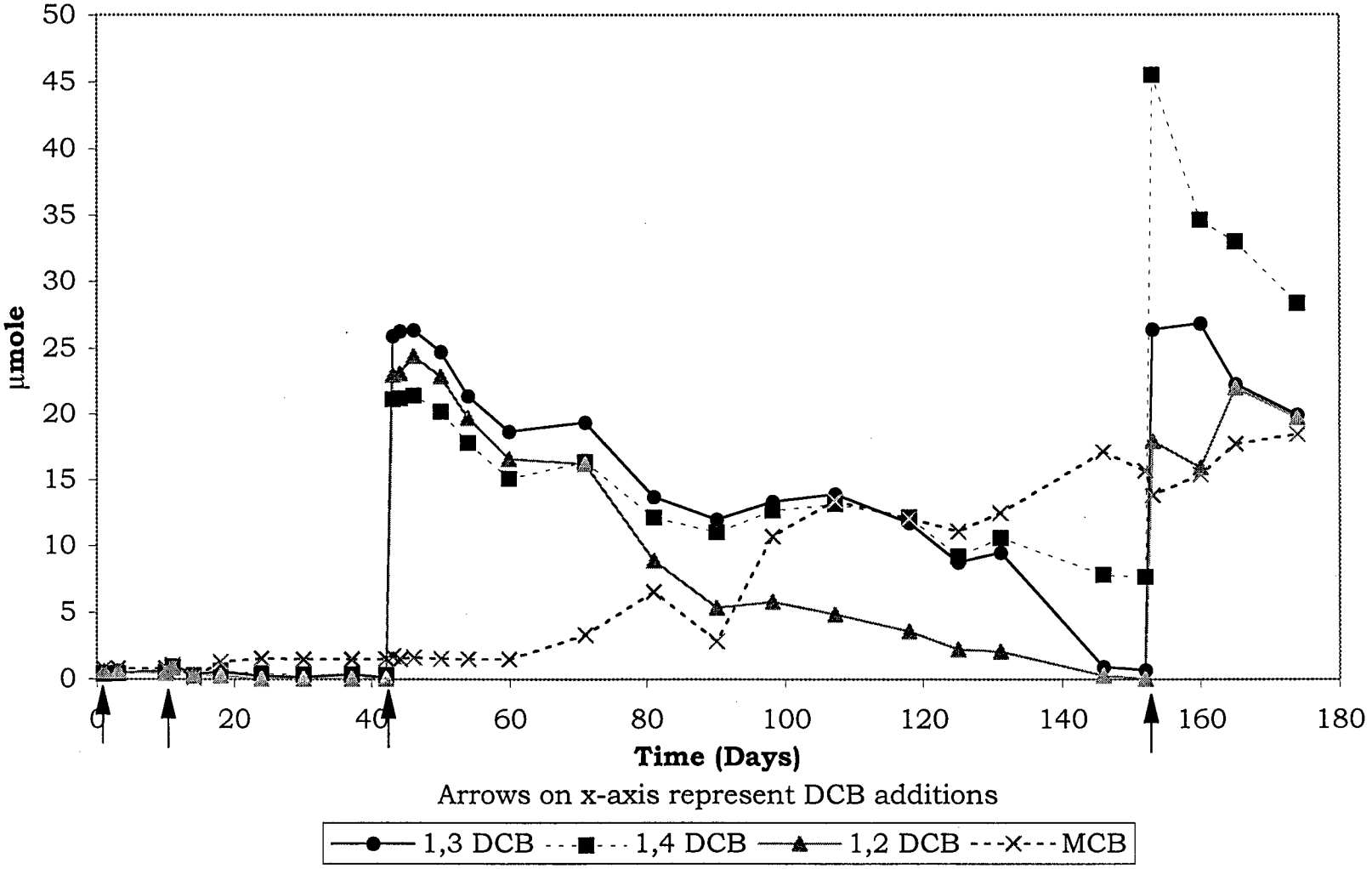
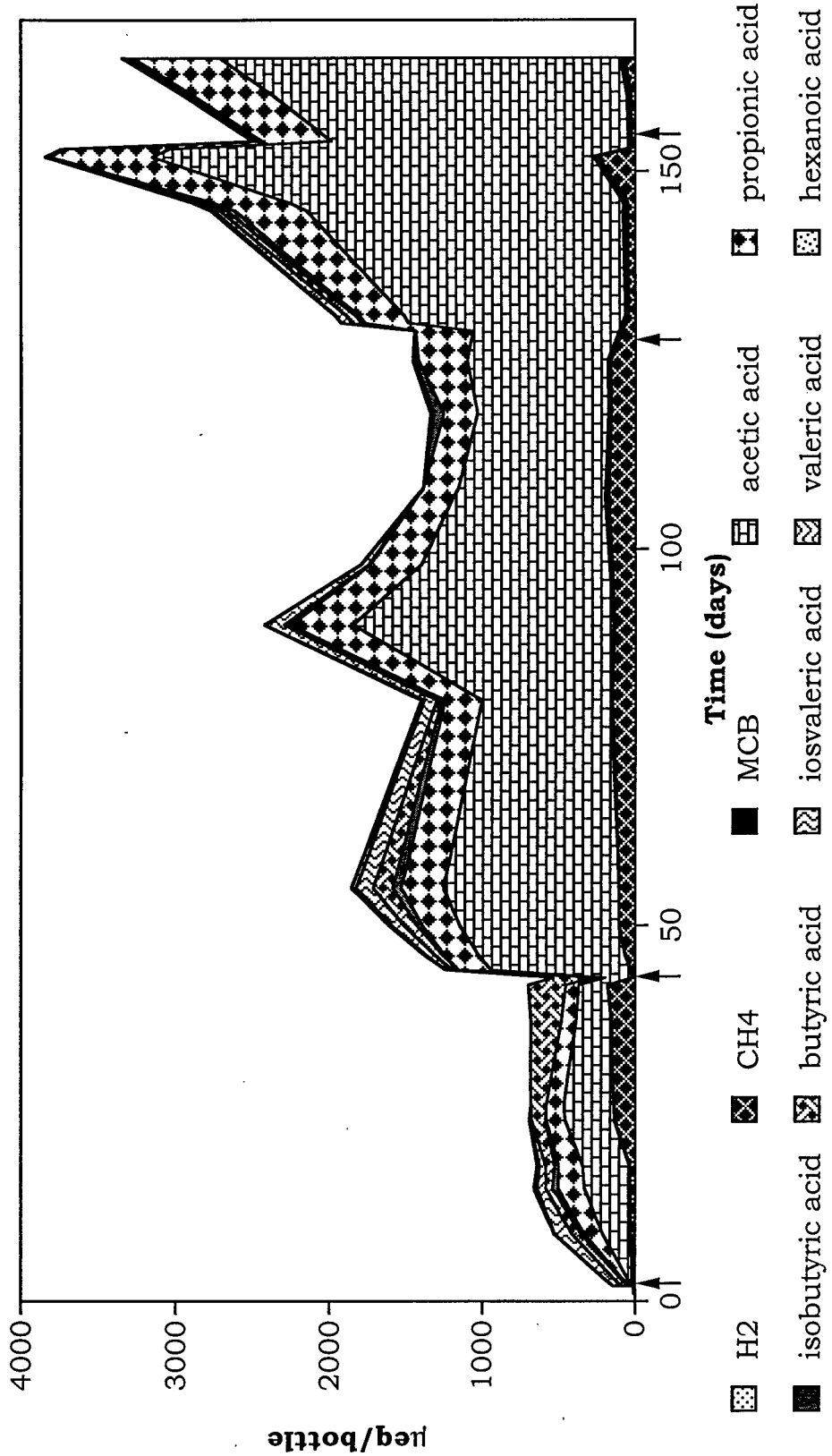


Figure 4.36 -- DCB and MCB Data for R-1-d Enrichment #4



Arrows on the x-axis represent YE additions

Figure 4.37 -- Equivalents Profile for R-1-d Enrichment #4

Table 4.18 -- R-1-s Sample Data

Moisture Content (% of wet weight)	11.26
Groundwater Alkalinity (meq/L)	9.1
Groundwater + Soil Alkalinity (meq/L)*	9.25
Conductivity (mS/cm)	937
Groundwater + Soil pH*	6.62

Table 4.19 -- R-1-s Background Data

	Background (µmole)
MCB	0.12
1,3-DCB	0
1,4-DCB	0
1,2-DCB	0

monitored throughout the experiment. Microcosms #1 through #9 were made with 50 g of soil and 92 g of groundwater and DCBs (8 g of the DCB stock 3.G.1a). Once dechlorination was observed, DCBs were again added. After sufficient data had been gathered for the natural-attenuation study, high levels of DCBs were added to several microcosms. The total levels of DCBs in the microcosms were the sums of the background and the added levels (Table 4.19, 4.20, 4.21).

Table 4.20 -- DCB Additions to R-1-s Microcosms #5 - #12

Microcosms		day 0	day 124	Total
#5 - #7	1,3-DCB	0.87	0.87	1.74
	1,4-DCB	0.85	0.85	1.70
	1,2-DCB	1.18	1.18	2.36
#8 - #12	1,3-DCB	0.87		0.87
	1,4-DCB	0.85		0.85
	1,2-DCB	1.18		1.18

In addition, microcosms #1 through #4 received 100 mg/L YE (0.2 ml from the YE stock 3.G.1c). Once VFA data confirmed that the

Table 4.21 -- DCB Additions to R-1-s Microcosms #1-#4. Additions Expressed in μmoles.

Microcosm		day 0	day 27	day 51	day 62	day 64	day 80	day 183	Total
#1	1,3-DCB	0.87	0.87	0.87	20.29			20.29	43.19
	1,4-DCB	0.85	0.85	0.85	18.37			20.41	41.33
	1,2-DCB	1.18	1.18	1.18	20.57			20.57	44.68
#2	1,3-DCB	0.87	0.87	0.87	20.29				22.90
	1,4-DCB	0.85	0.85	0.85	20.41				22.96
	1,2-DCB	1.18	1.18	1.18	20.57				24.11
#3	1,3-DCB	0.87		0.87	0.87		0.87	20.29	23.77
	1,4-DCB	0.85		0.85	0.85		0.85	19.73	23.13
	1,2-DCB	1.18		1.18	1.18		1.18	20.57	25.29
#4	1,3-DCB	0.87		0.87		0.87	0.87		3.48
	1,4-DCB	0.85		0.85		0.85	0.85		3.4
	1,2-DCB	1.18		1.18		1.18	1.18		4.72

electron donor had been consumed, more YE was added. Table 4.21 contains the YE-addition data. Microcosms #1 and #2 received an

Table 4.22 -- YE Additions to R-1-s Microcosms

Microcosms	day 0	day 52	day 64	day 81	day 148	day 184
#1	X	X	X			X
#2	X	X	X		X	
#3	X	X		X		X
#4	X	X		X		

addition of $NaHCO_3$ (bringing the total alkalinity up to 50 meq/L) on day 62, and microcosm #3 received an addition of $NaHCO_3$ on day 183. Microcosms #8 and #9 were autoclaved. Microcosms #10 through #12 were made with 100 ml of distilled water, DCBs (8 g from the DCB stock) and were autoclaved (Table 4.20).

The first DCB and MCB data points were taken on day 1. All the microcosms were run for 217 days. Dechlorination was evident after 12 days. The DCB and MCB profiles for fed microcosm #1 through #4 are represented in Figures 4.38, 4.39, 4.40 and 4.41. The VFA levels (and equivalents profiles) for microcosms #1 and #2 are represented in Figure 4.42 and 4.43. The MCB accumulation in microcosm #1 through #4 accounted for 34%, 6%, 6% and 44% of the total (initial plus added) DCBs, respectively (but 42%, 7%, 6% and 100% of the total recovered chlorinated benzenes) . Benzene formation was only noted in microcosm #1 (Figure 4.44). The benzene accumulation in microcosm #1 accounted for 1% of the recovered MCB.

The DCB and MCB level profiles for unfed microcosms #5 through #7 were similar to each other and are represented in by microcosm #7 in Figure 4.45. The MCB accumulation in microcosm

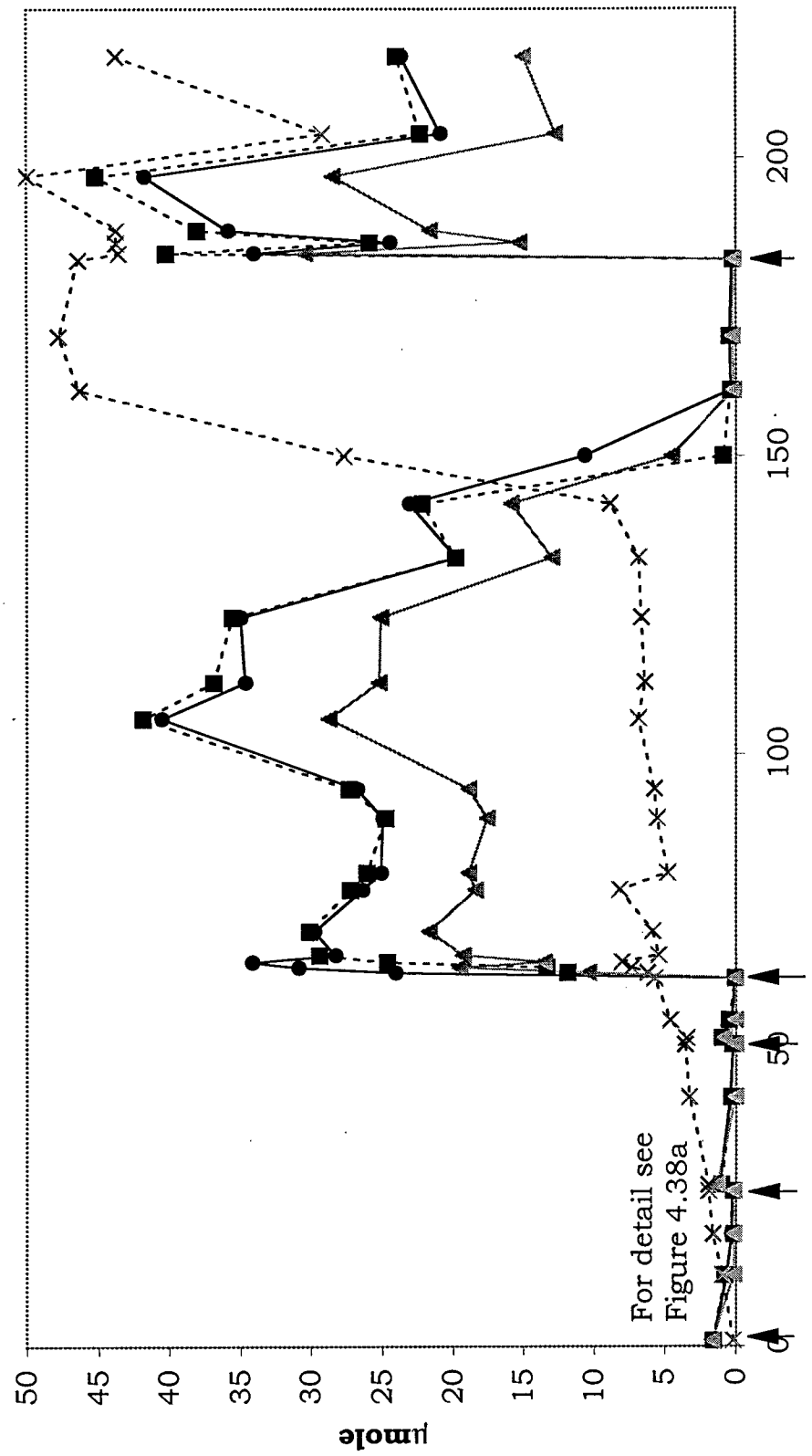


Figure 4.38 -- DCB and MCB Concentrations for R-1-s Microcosm #1 (Fed)

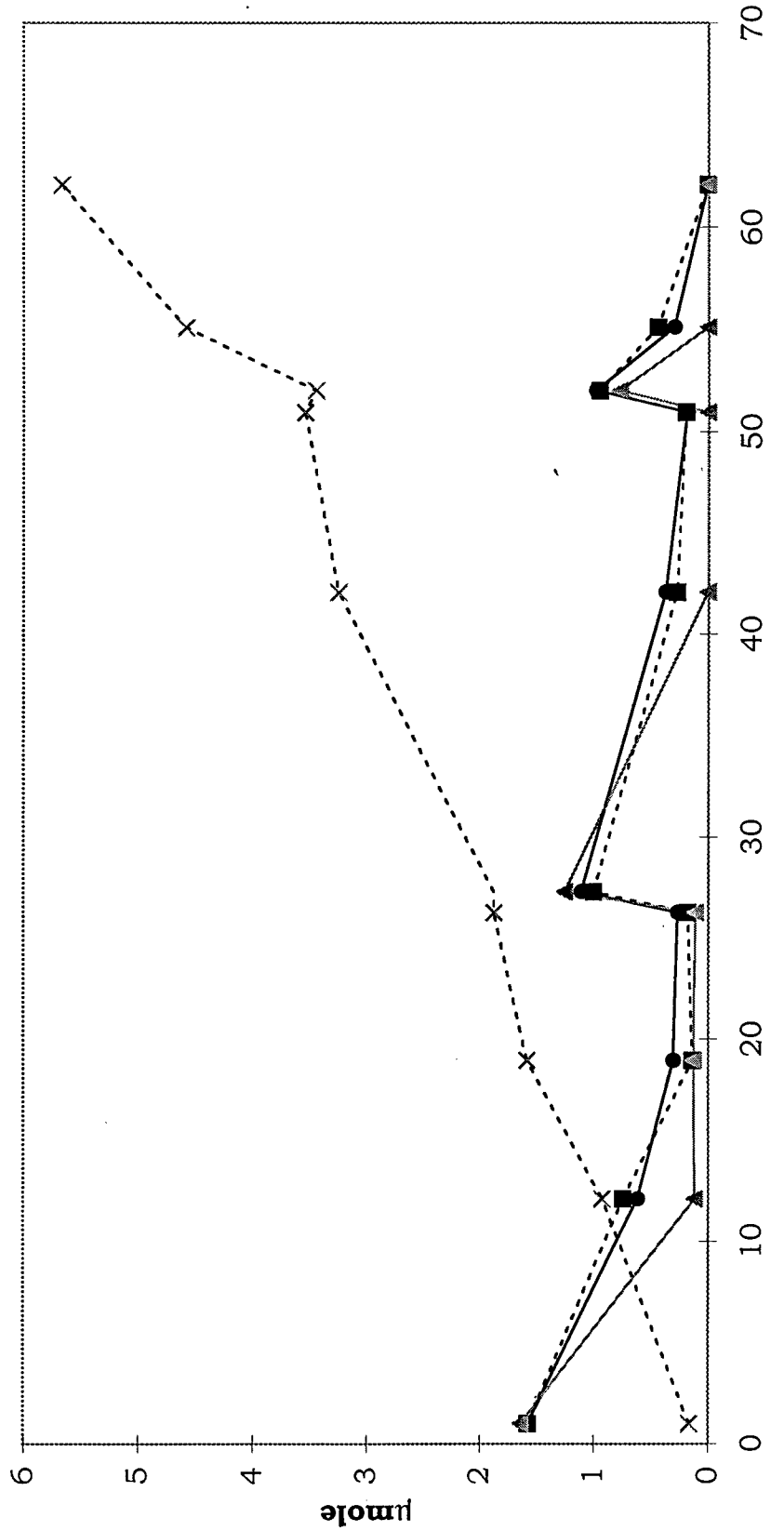


Figure 4.38a -- DCB and MCB Concentrations for R-1-s Microcosm #1 (Fed) Detail

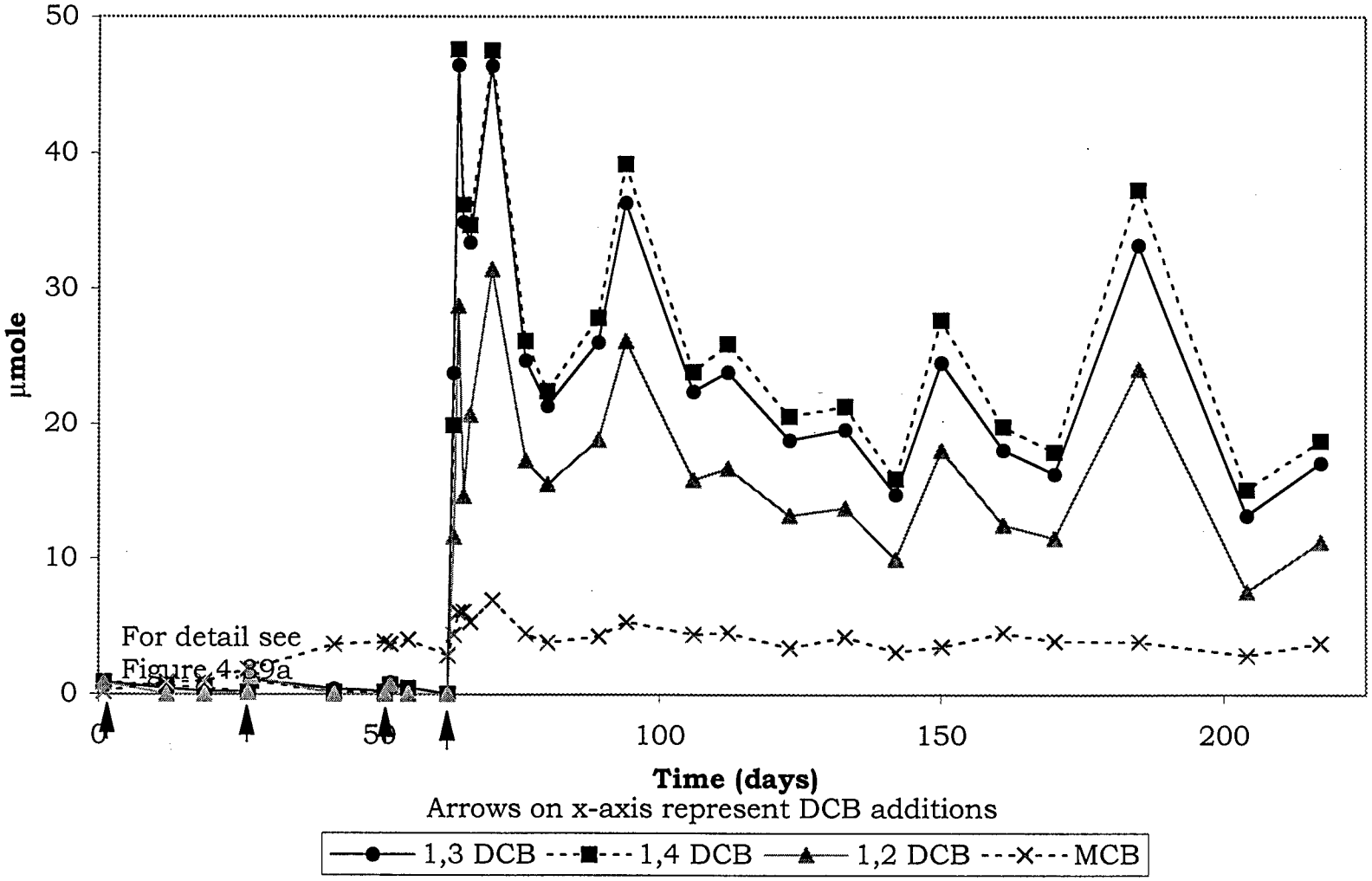


Figure 4.39 -- DCB and MCB Concentrations for R-1-s Microcosm #2 (Fed)

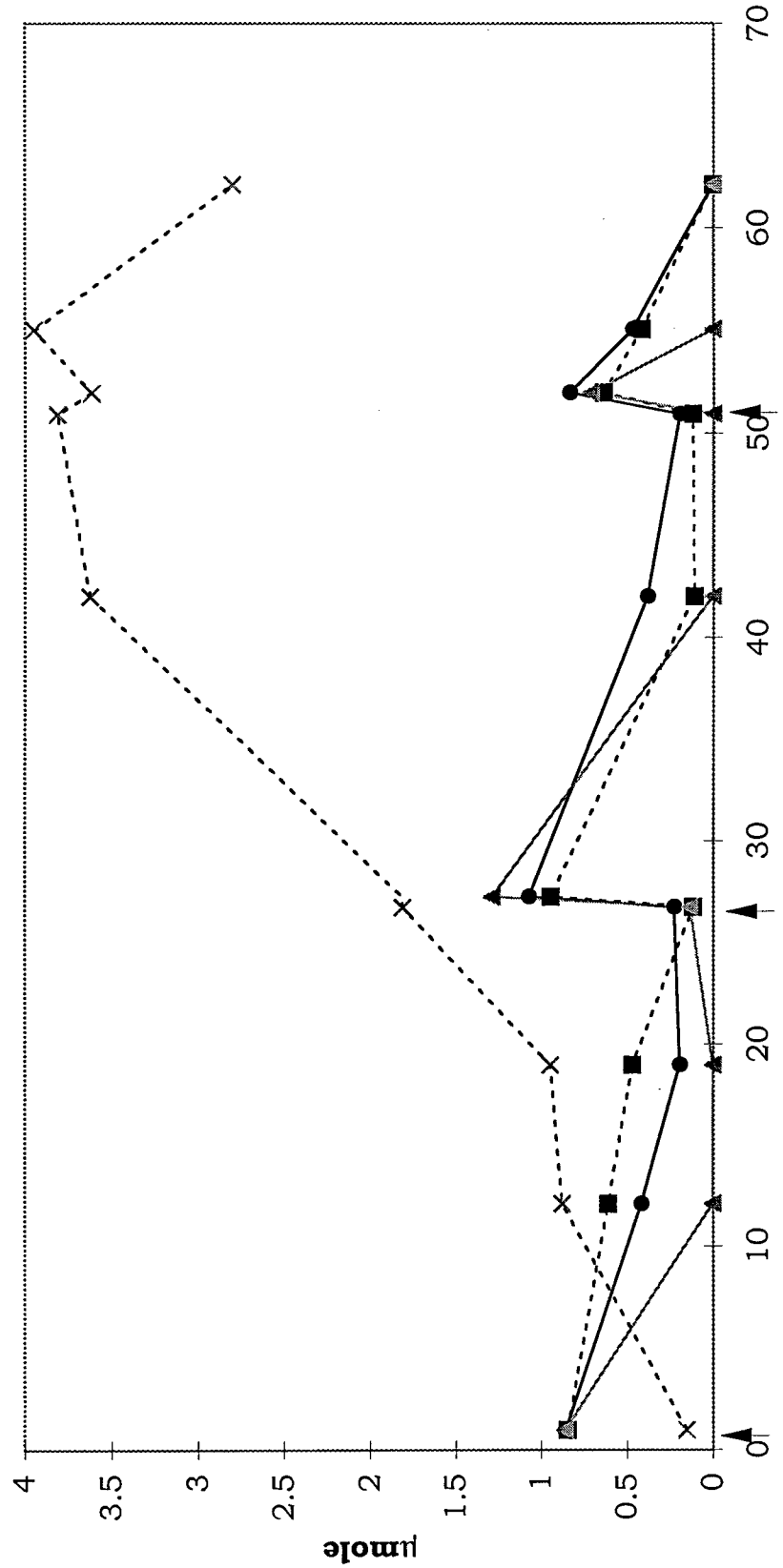


Figure 4.39a -- DCB and MCB Concentrations for R-1-s Microcosm #2 (Fed) Detail

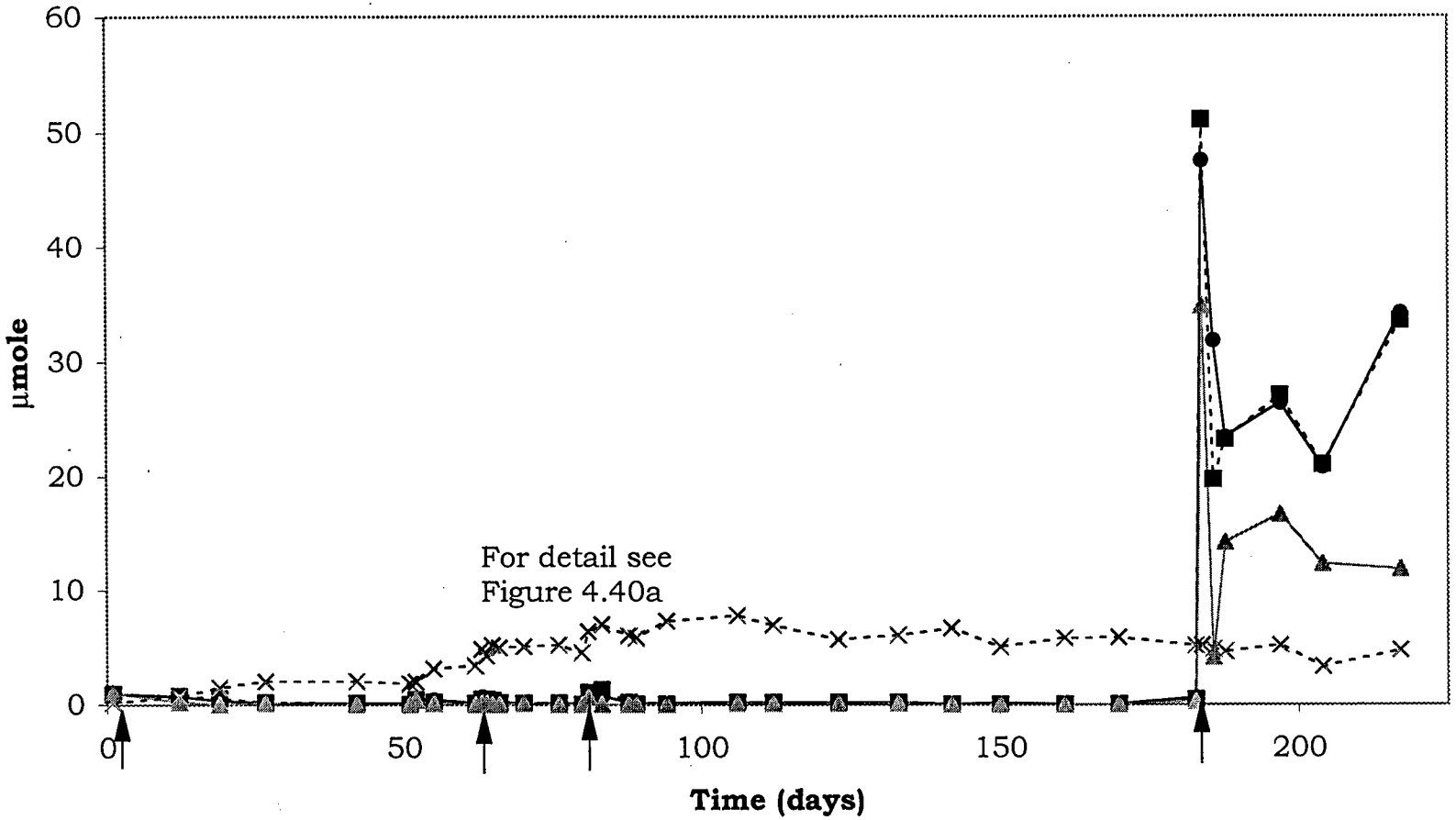


Figure 4.40 -- DCB and MCB Concentrations for R-1-s Microcosm #3 (Fed)

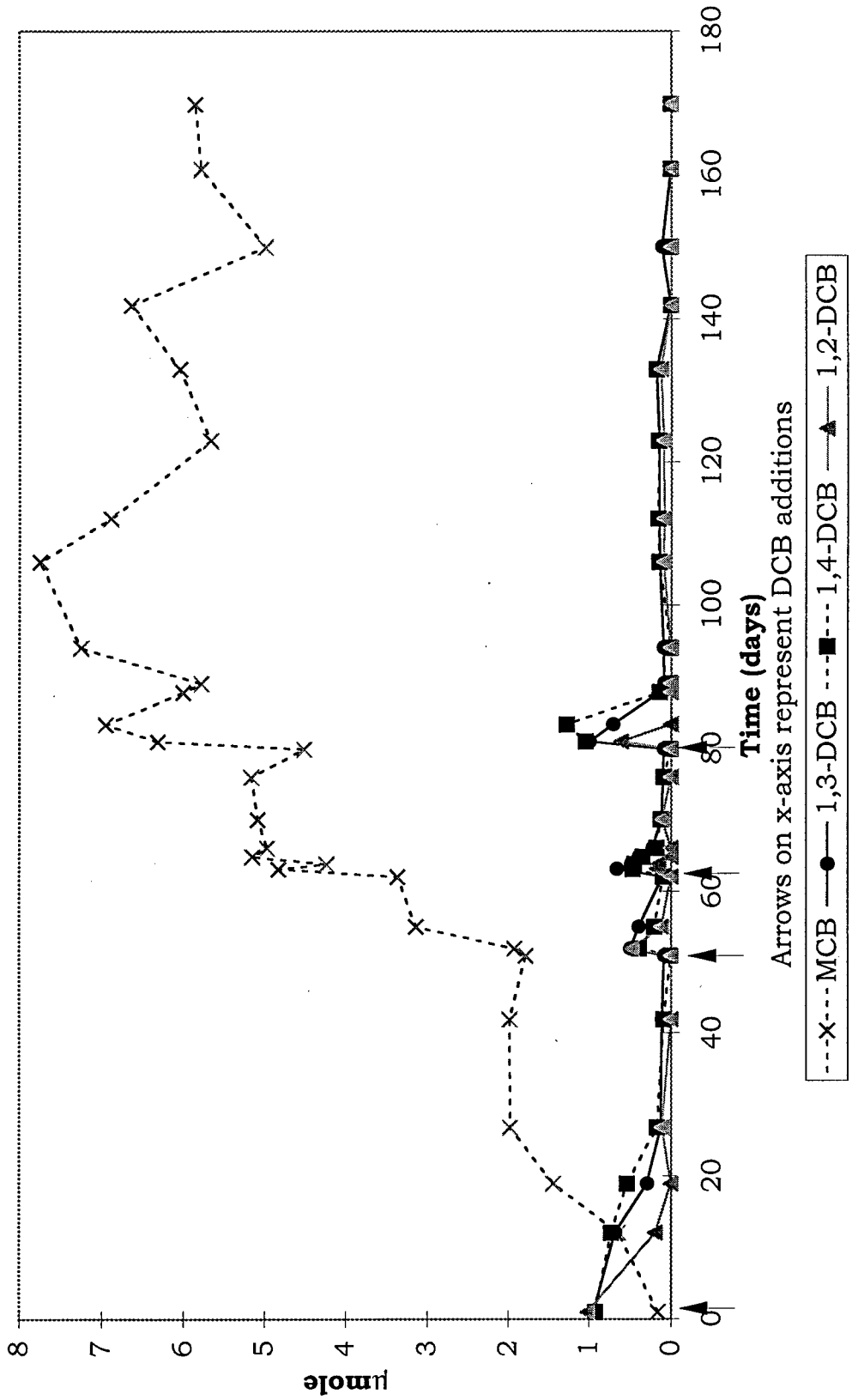


Figure 4.40a -- DCB and MCB Concentrations for R-1-s Microcosm #3 (Fed) Detail

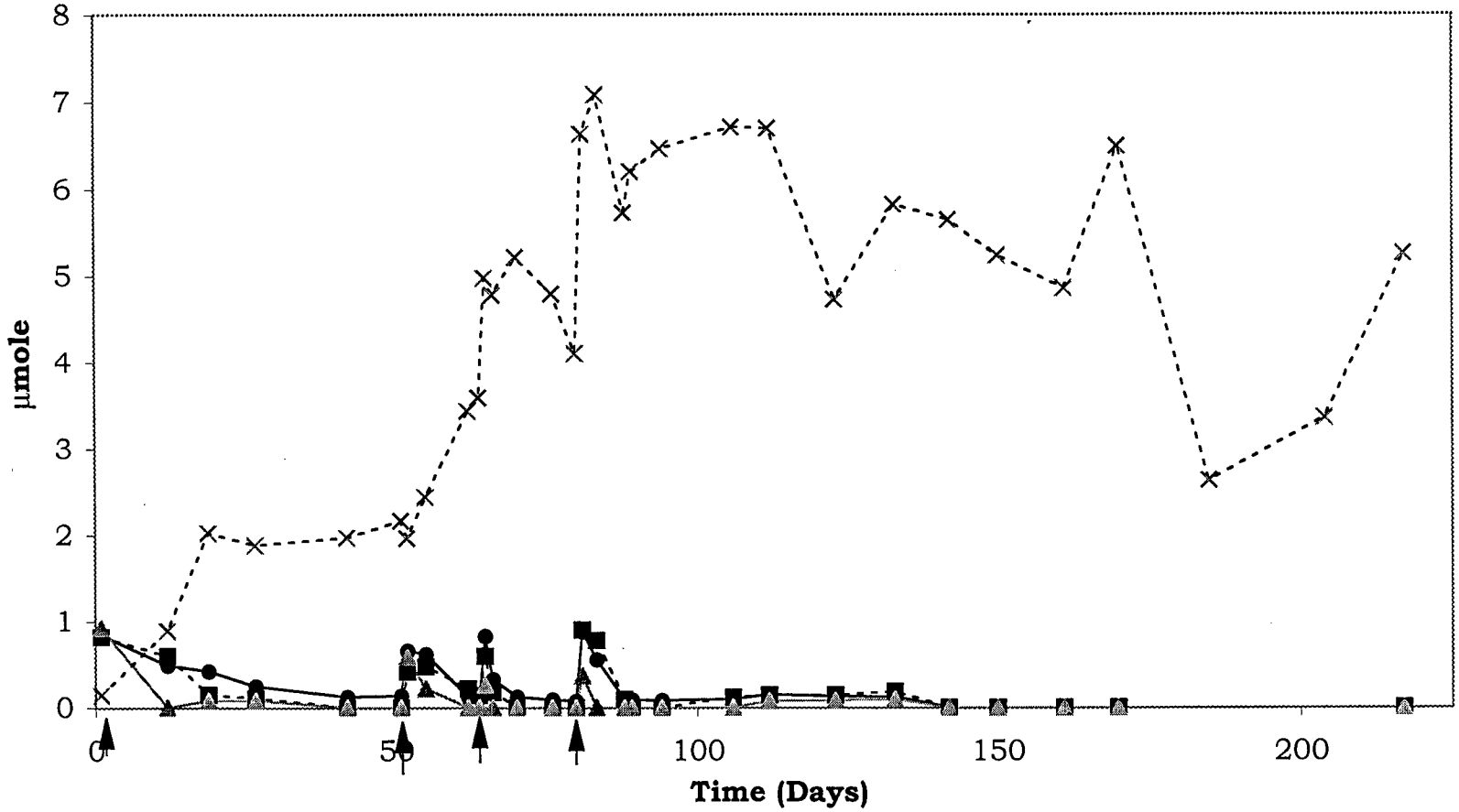
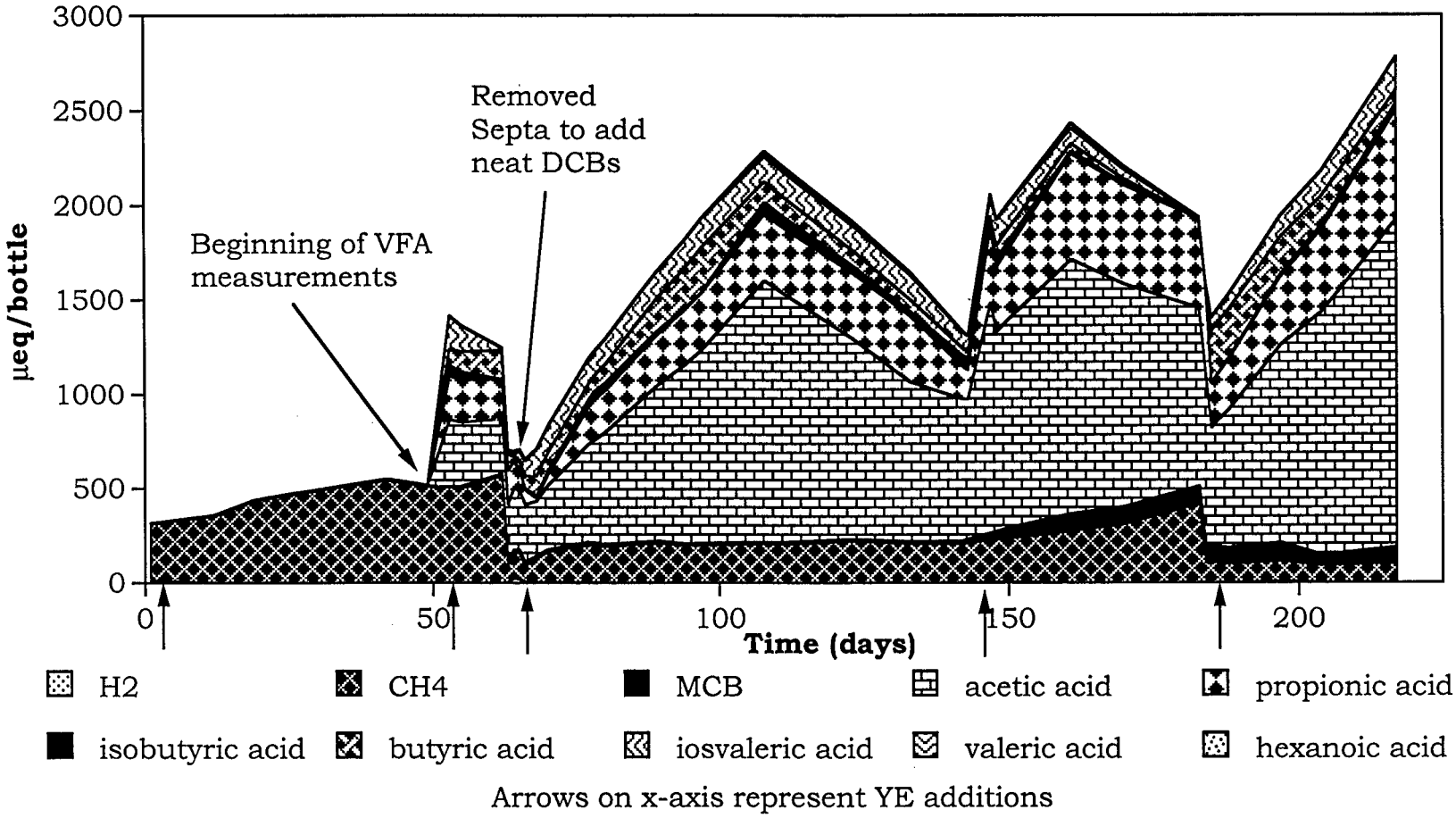
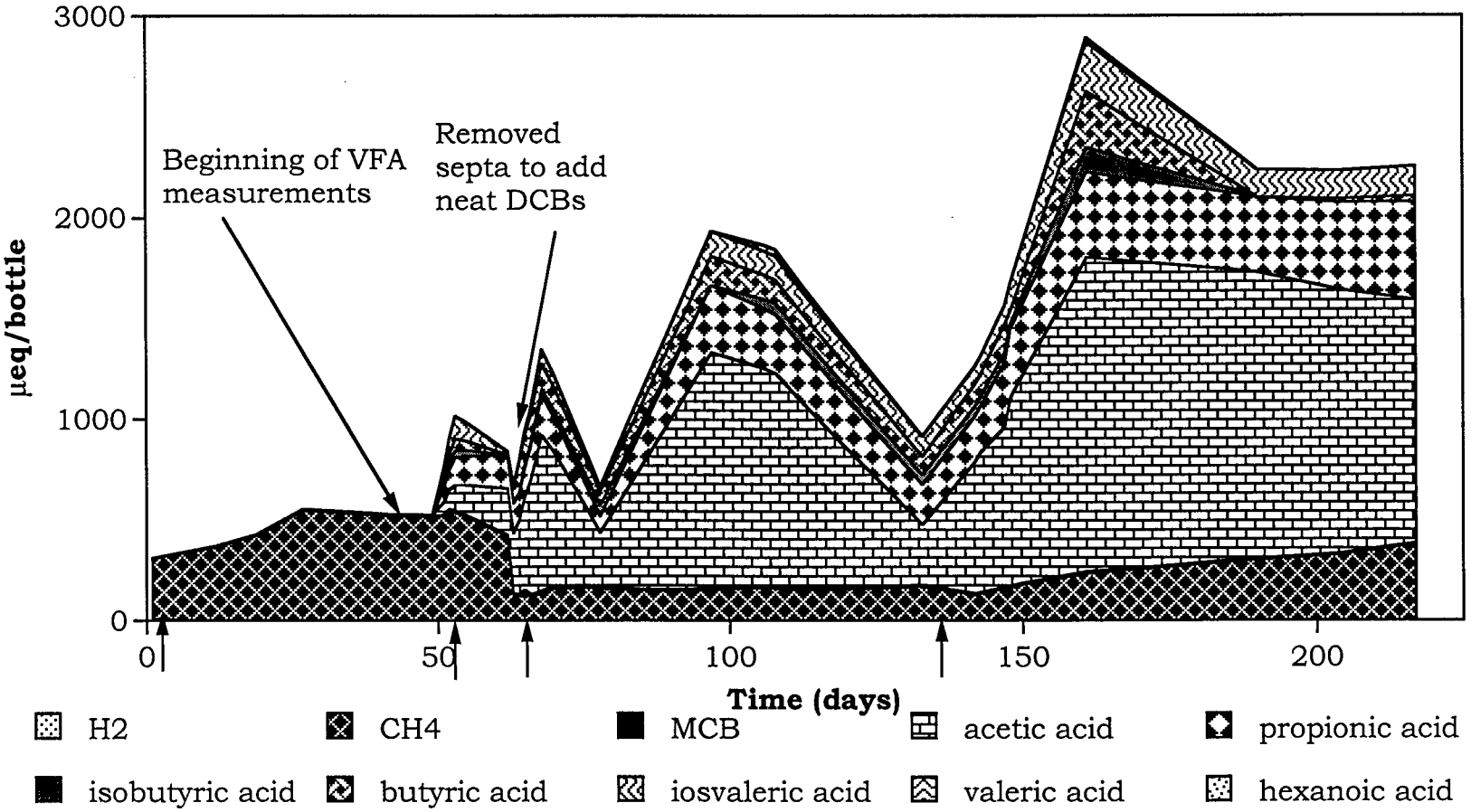


Figure 4.41 -- DCB and MCB Data for R-1-s Microcosm #4 (Fed)



Arrows on x-axis represent YE additions

Figure 4.42 -- Equivalents Profile for R-1-s Microcosm #1 (Fed)



Arrows onx-axis represent YE additions

Figure 4.43 -- Equivalents Profile for R-1-s Microcosm #2

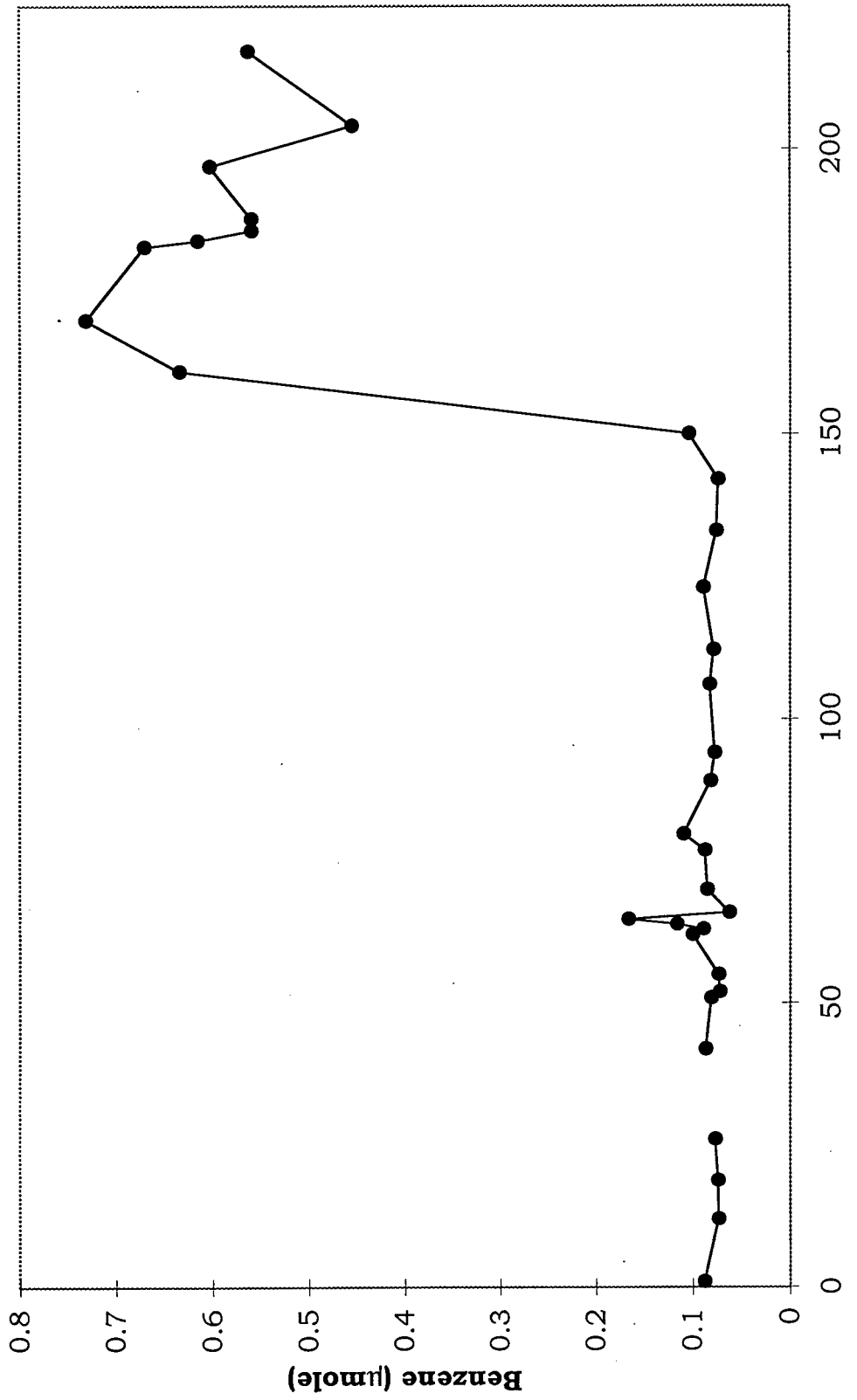


Figure 4.44 -- Benzene Concentrations for R-1-s Microcosm #1

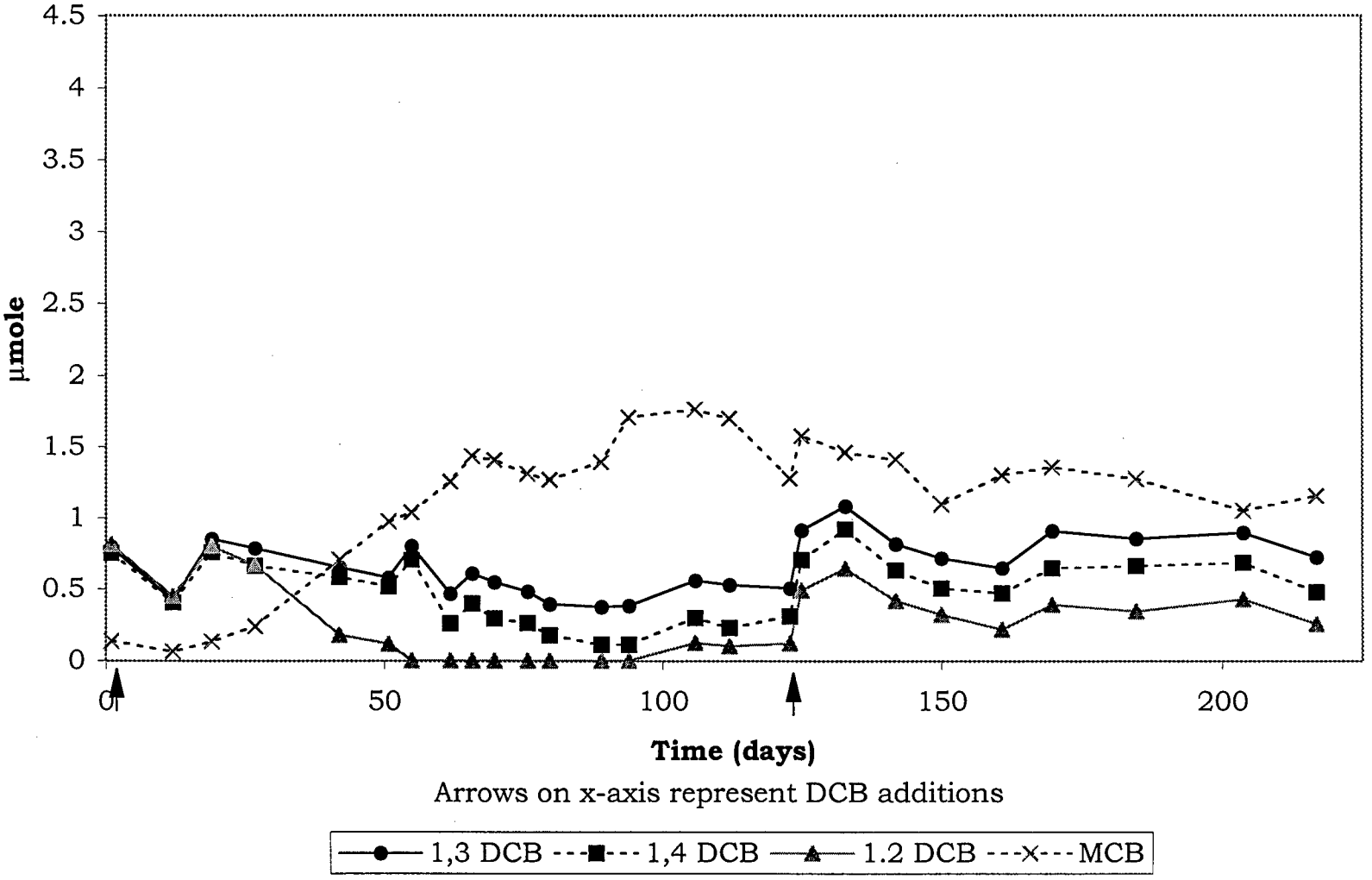


Figure 4.45 -- DCB and MCB Concentrations for R-1-s Microcosm #7 (Unfed)

#7 accounted for 25% of the total (initial plus added) DCBs (but 44% of the total recovered chlorinated benzenes).

The DCB and MCB profiles for autoclaved microcosms #8 and #9 were similar and are represented in Figure 4.46. The DCB and MCB profiles for water-control microcosms #10 through #12 were similar to Figure 4.3.

On January 14, 1999, four enrichments were prepared in 94 ml of basal medium. Enrichments #1 through #4 were made with 8 ml inocula of the silty liquid from microcosms #1 through #4 respectively. Enrichments #1 through #4 also received 8 ml of DCB stock and 0.2 ml of YE stock. In addition, enrichments #3 and #4 received another 8-ml inocula from microcosms #3 and #4 eleven days later, on January 25, 1999. After the DCBs and YE had been consumed, more DCBs and YE were added. Table 4.23 and 4.24 detail the DCB and YE additions.

Table 4.23 -- DCB Additions to R-1-s Enrichments.
(Additions Expressed in µmoles.)

Enrichments		day 0	day 43	day 132	Total
#1	1,3-DCB	0.87		0.87	1.74
	1,4-DCB	0.85		0.85	1.70
	1,2-DCB	1.18		1.18	2.36
#2	1,3-DCB	0.87		0.87	1.74
	1,4-DCB	0.85		0.85	1.70
	1,2-DCB	1.18		1.18	2.36
#3	1,3-DCB	0.87	20.29		21.16
	1,4-DCB	0.85	21.77		22.62
	1,2-DCB	1.18	20.57		21.75
#4	1,3-DCB	0.87	20.29		21.16
	1,4-DCB	0.85	20.41		21.26
	1,2-DCB	1.18	20.57		21.75

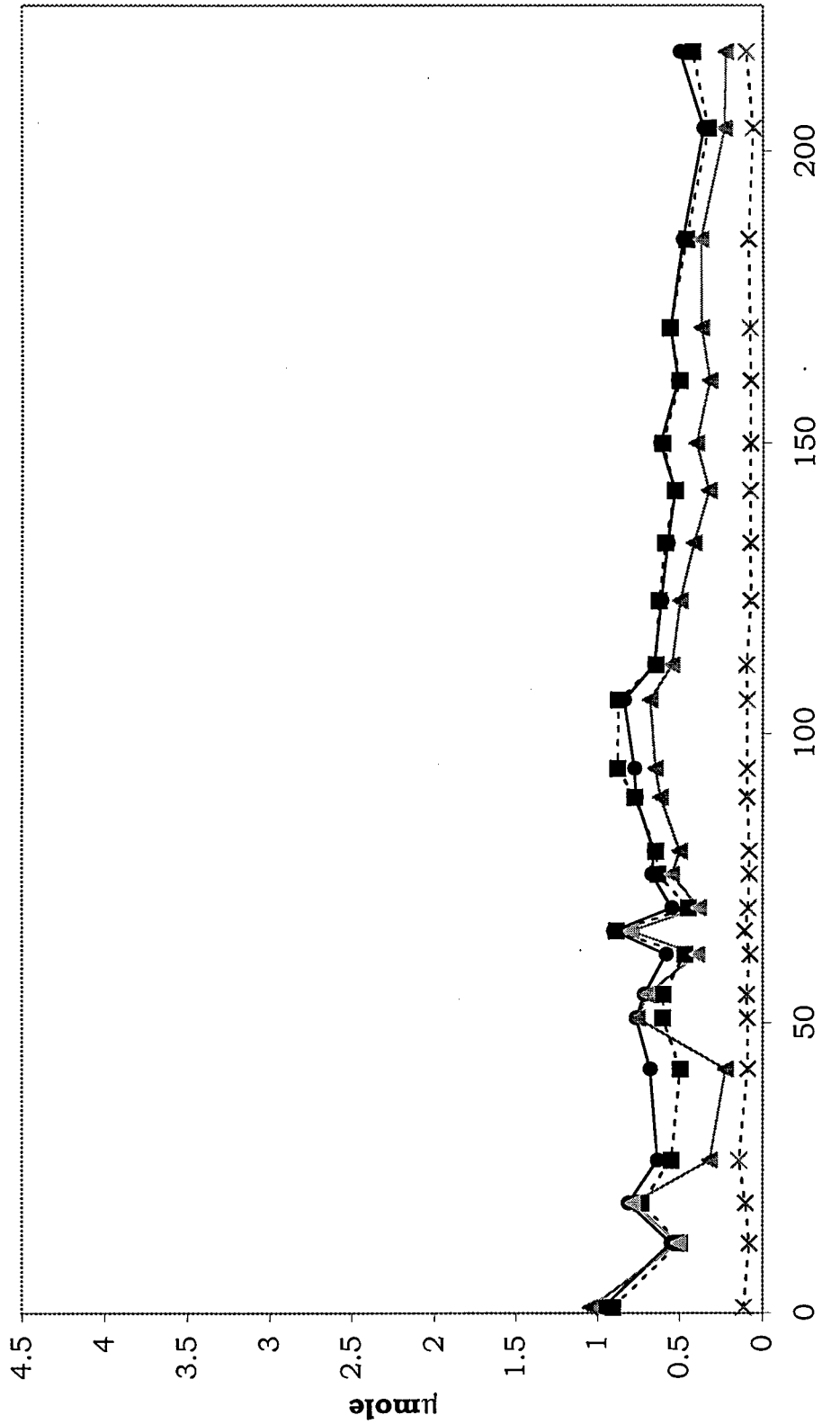


Figure 4.46 -- DCB and MCB Data for R-1-s Microcosm #9 (Autoclaved Control)

Table 4.24 -- YE Additions to R-1-s Enrichments

Enrichment	day 0	day 11	day 44	day 97	day 133
#1	X			X	X
#2	X				X
#3	X	X	X	X	
#4	X		X	X	

The first DCB and MCB data points were taken on day 1. All the enrichments were run for 166 days. Dechlorination was evident after 15 days for enrichments #3 and #4 and after 40 days for enrichments #1 and #2. The DCB and MCB profiles and equivalents profiles for enrichments #1 and 2 were similar and are represented by enrichment #1 in Figures 4.47 and 4.48 respectively. The MCB accumulation in enrichment #1 accounted for 7% of the total (initial plus added) DCBs (but 64% of the total recovered chlorinated benzenes).

The DCB and MCB profiles and the equivalents profiles for enrichment #3 are represented in Figures 4.49 and 4.50, respectively. The MCB accumulation in enrichment #3 accounted for 1% of the total (initial plus added) DCBs (but 4% of the total recovered chlorinated benzenes).

The DCB and MCB profiles and the equivalents profiles for enrichment #4 are represented in Figures 4.51 and 4.52 respectively. The MCB accumulation in enrichment #4 accounted for 12% of the total (initial plus added) DCBs (but 24% of the total recovered chlorinated benzenes).

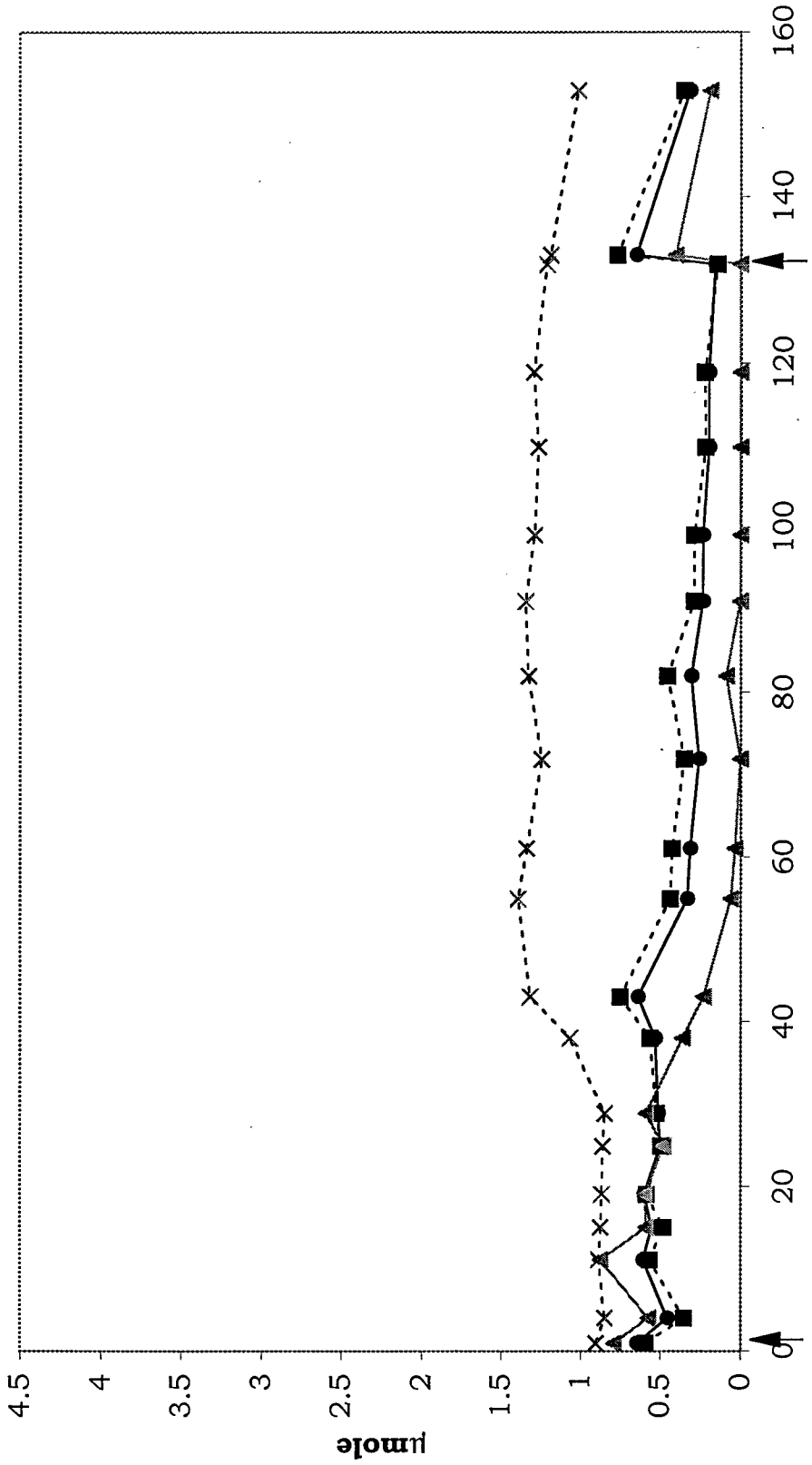
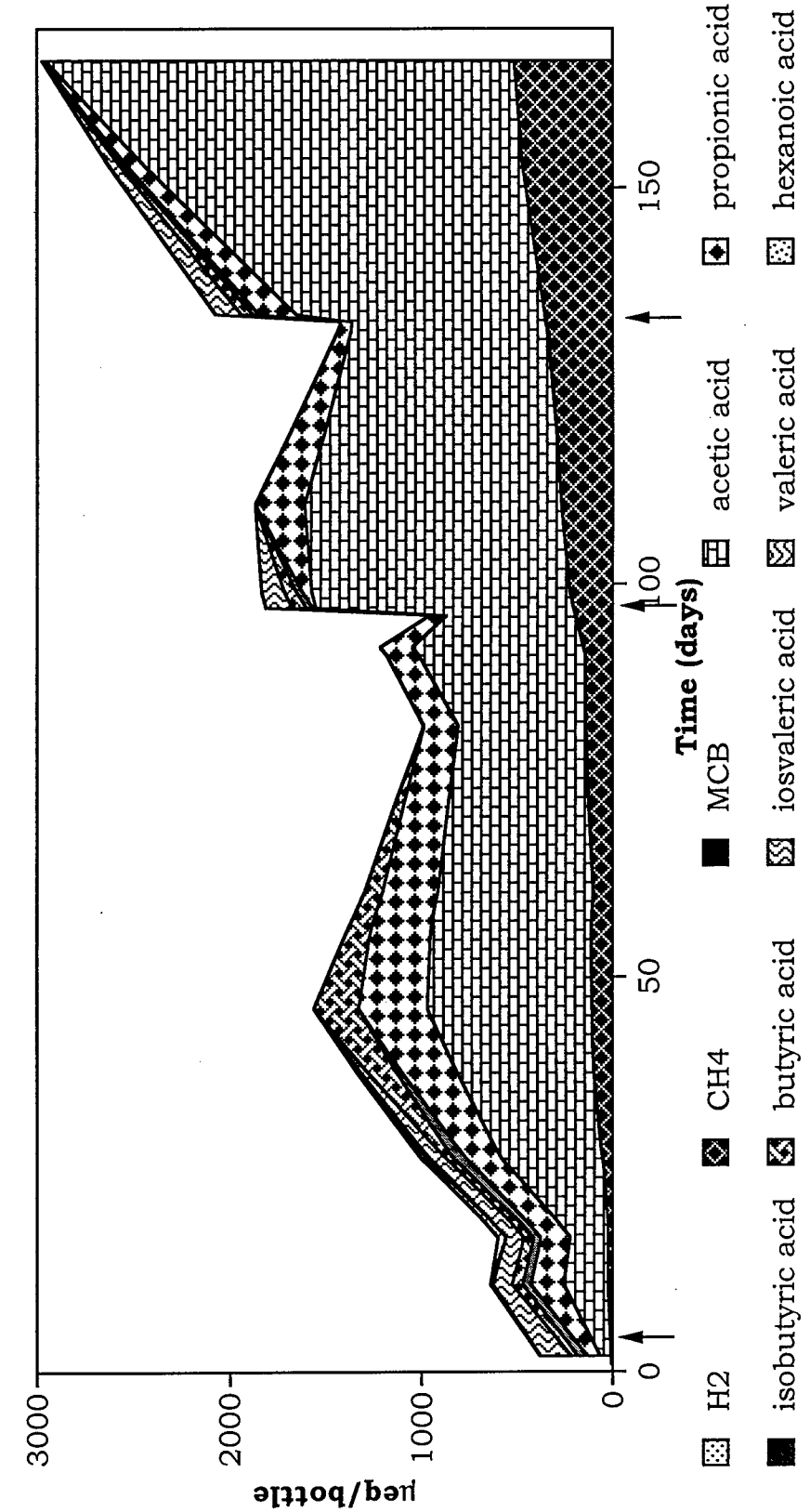


Figure 4.47 -- DCB and MCB Concentrations for R-1-s Enrichment #1



Arrows on the x-axis represent YE additions

Figure 4.48 -- Equivalents Profile for R-1-s Enrichment #1

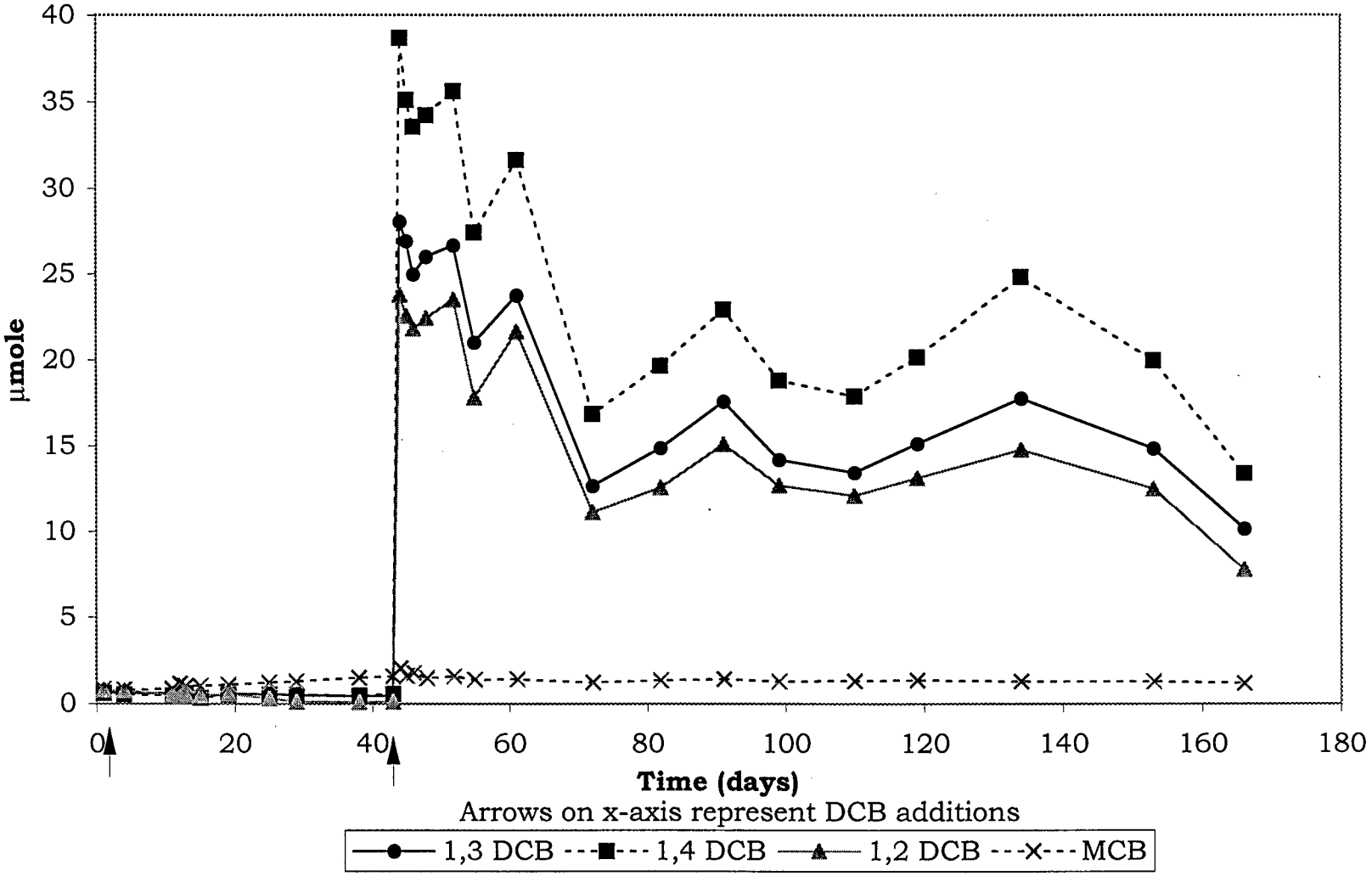
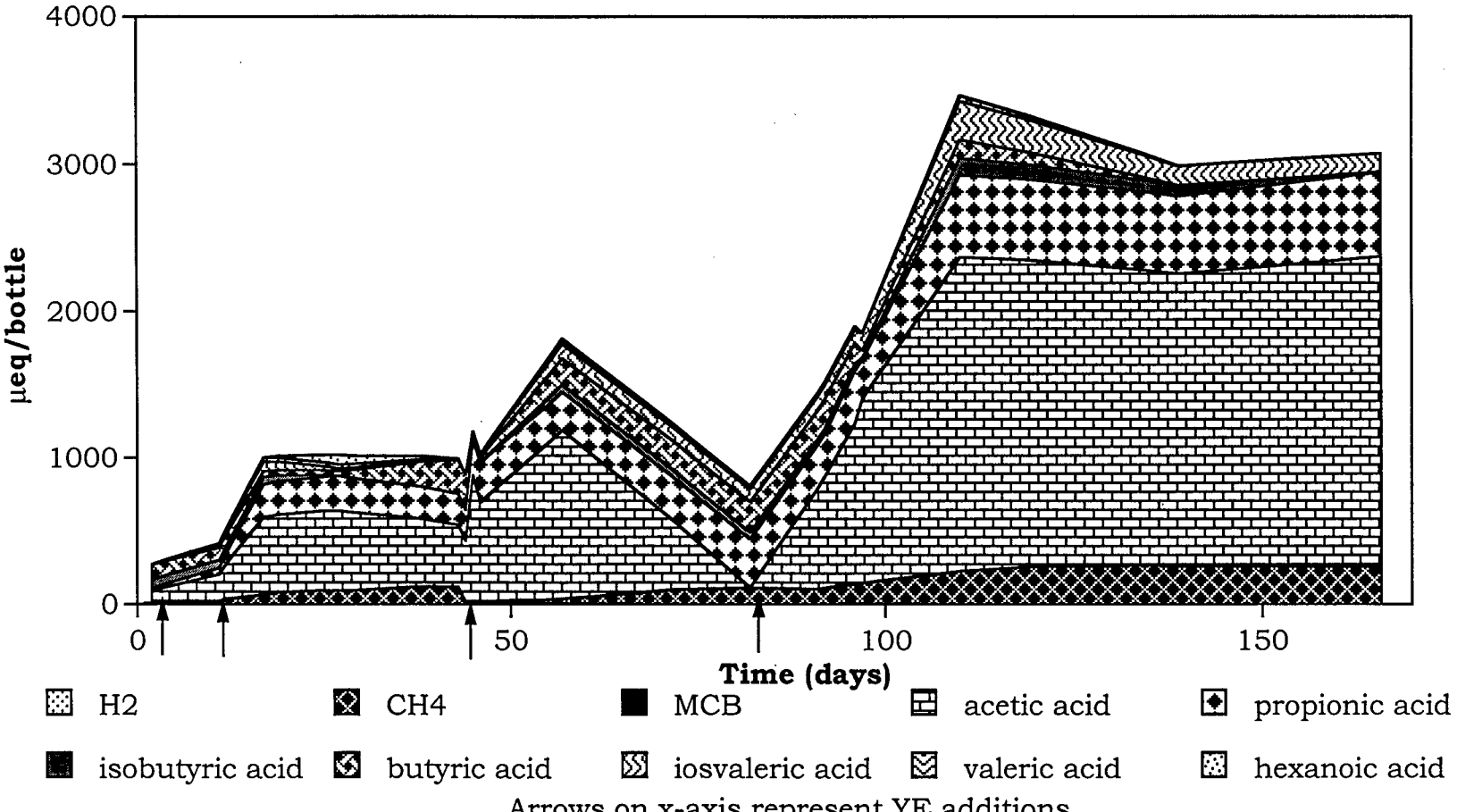


Figure 4.49 -- DCB and MCB Concentrations for R-1-s Enrichment #3



Arrows on x-axis represent YE additions

Figure 4.50 -- Equivalents Profile for R-1-s Enrichment #3

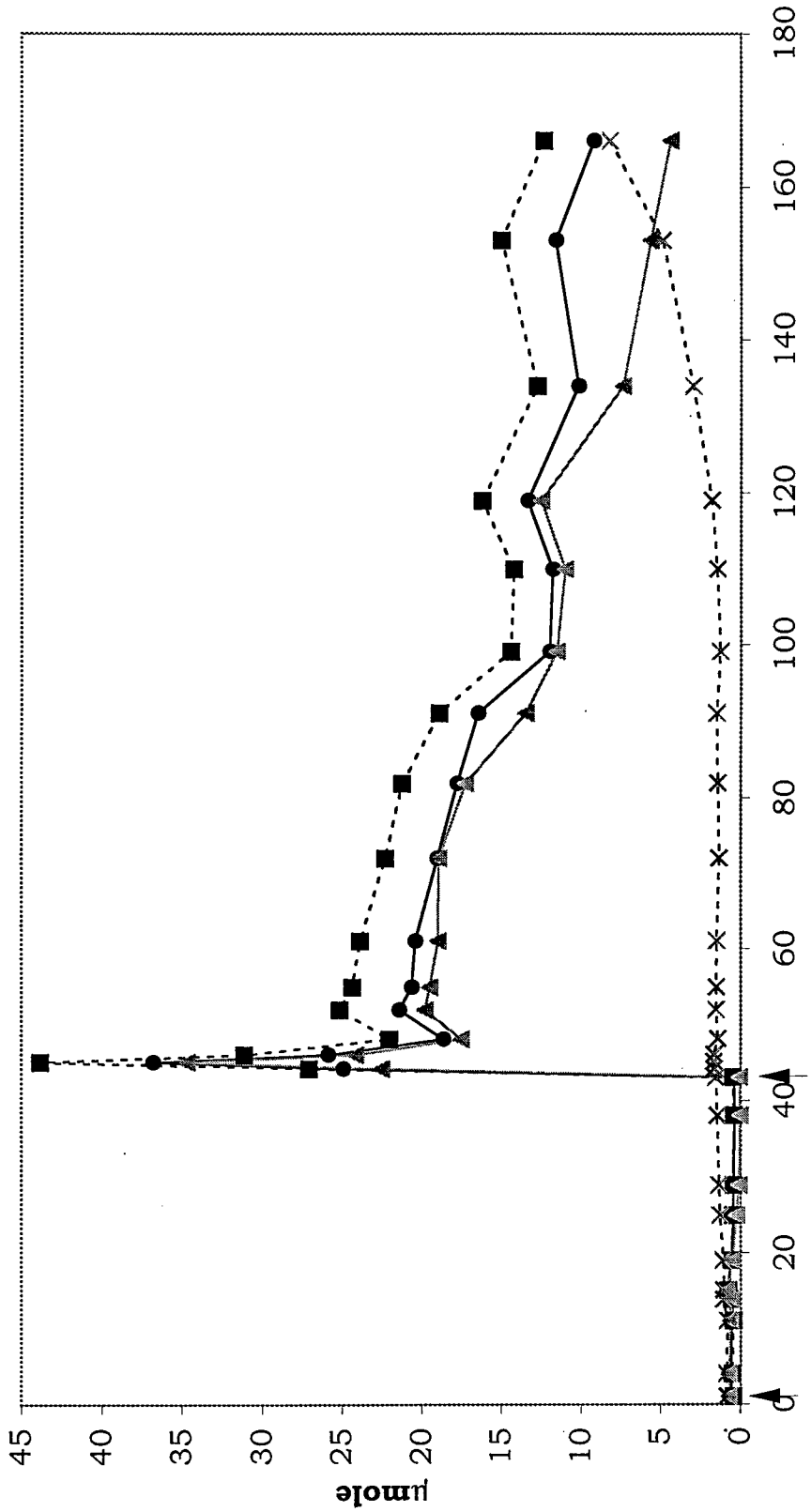
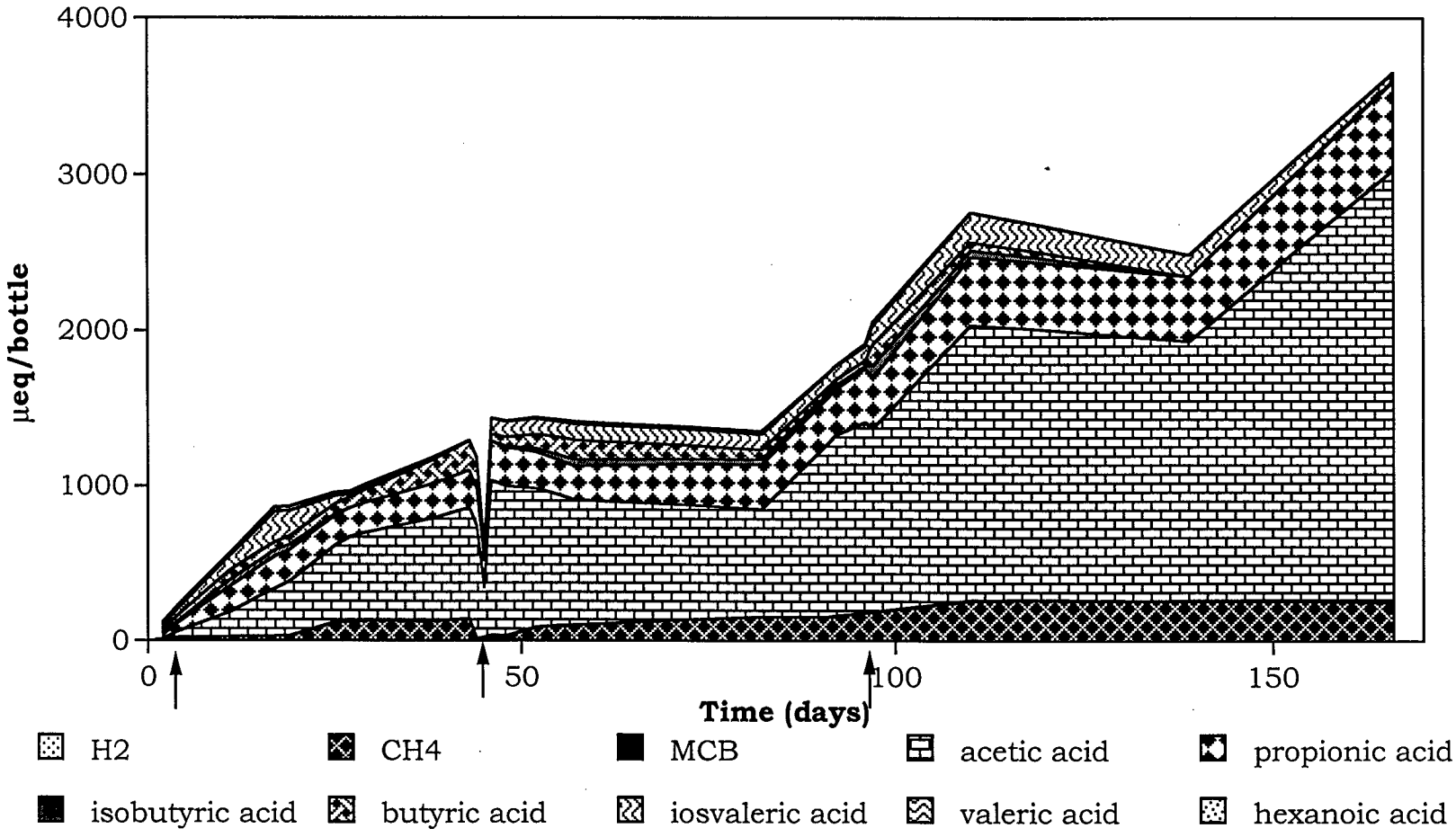


Figure 4.51 -- DCB and MCB Concentrations for R-1-s Enrichment #4



Arrows on x-axis represent YE additions

Figure 4.52 -- Equivalents Profile for R-1-s Enrichment #4

CHAPTER FIVE -- DISCUSSION

5.A Plattsburgh Air Force Base

None of the P microcosms formed any MCB, nor showed evidence of DCB loss unaccounted for in controls. This could be because anaerobic DCB-degrading microorganisms were not present in the material obtained from the site.

Another reason for the lack of DCB dechlorinator growth could be that high levels of ethanol present in the glovebox hindered the DCB dechlorinators. Initially ethanol was used to sanitize the glovebox in-between uses. (This problem was corrected after the setup of this site). The high levels of ethanol did not hinder all cell growth, because methanogens were active (i.e., high levels of CH_4 were formed during the experiment). In addition, the TCE originally present in the active microcosms disappeared. An increase in vinyl-chloride was not noted, but the TCE could have been converted to ethene. (Ethene was not monitored). Since the same TCE decrease did not occur in the control microcosms, it is possible that TCE dechlorinators were active in these microcosms.

5.B Robins Air Force Base at Well R13-2W

No MCB production was noted in any R-2 microcosms, nor showed evidence of DCB loss above losses in controls. This could be because the conditions at the site were rather unfavorable for microbial growth (pH of 5.5 and alkalinity of <0.25 meq/L). There was some microbial activity in the beginning, since the H_2 present in the

microcosms at setup was consumed in the active microcosms by the first sampling period (7 days). However, CH_4 levels did not change appreciably in any microcosm.

5.C *Robins Air Force Base Landfill Leachate*

No MCB production was noted in any R-LF microcosms, nor did DCB losses exceed those in the controls. This could be for the same reasons stated in Section 5.B. In addition, the leachate sample was not very reduced. All the microcosms were pink (from the resazurin) throughout the experiment. The fact that highly reduced conditions failed to develop, even in YE-fed microcosms, further supports the existence of unsuitable conditions.

5.D *Digested Sludge from the Ithaca Wastewater Treatment Plant*

Slow MCB production was evident after about 250 days coincident with slow 1,2-DCB degradation. The MCB production accounted for at most 10% of the total DCBs and 12% of the total recovered chlorinated benzenes.

DCB dechlorination was probably not occurring significantly at the treatment plant, as the digested sludge contained no measurable DCBs or MCB. The onset of DCB dechlorination in microcosms could be because microbes present in the sludge, over time, adapted, and by day 250 could gain energy from the dechlorination of DCBs. Another explanation (although not as likely) for the appearance of MCB could be that a population of a organism was slowly growing

that incidentally produced MCB while metabolizing other substrates (co-metabolism).

These microcosms will continue to be monitored.

5.E Kelly Air Force Base

Although there were analytical difficulties due to the LNAPL present in the sample, MCB production did seem to be occurring in the K microcosms. Dechlorination of 1,2-DCB was slightly favored over 1,3-DCB and both were much favored over the dechlorination of 1,4-DCB. In some microcosms it was possible that 1,4-DCB dechlorination was not occurring at all (Figures 4.8, 4.9 and 4.12). This finding is in agreement with Bosma et al. They stated that since the dechlorination proceeded first by the addition of an electron, and that the electron addition could be balanced by the electronegativity of the chlorine substituents, dechlorination would be favored for molecules with nearby chlorine substituents (Bosma 1988).

In the microcosms fed DCBs a second time, the rate of dechlorination substantially increased. This suggests that the microbes dechlorinating the DCBs were using DCBs as metabolic substrates. MCB was the only tested byproduct of the DCB dechlorination.

DCB dechlorination did occur in the bottles not fed an external electron donor. It is difficult to ascertain the significance of DCB dechlorination that might be naturally occurring at Kelly Air Force

Base, since the microcosms were perturbed from their native state (purged for eight hours and given unnaturally high levels of DCBs).

The fact that the re-made microcosms (#2, #3 and #6, Figure 4.11) differed significantly from the earlier-prepared microcosms is perplexing. It could be that during the initial microcosm setup, the soil samples were not well-mixed and the soil left behind differed significantly from the soil used in the initial microcosms. This explanation seems reasonable since the appearance of the re-made microcosms differed from that of the initial microcosms. When the re-made microcosms were agitated, they settled immediately into a clear supernatant and the soil and rocks, whereas the initial microcosms took several days to settle.

This difficulty in completely mixing the soil samples could have also caused differences in the sorptive capabilities of the individual microcosms -- especially given the LNAPL phase associated with the Kelly Air Force Base material. This could explain the differences in initial measured values for microcosms #1 (Figure 4.8), #4 (Figure 4.9) and #5 (Figure 4.10).

The MCB production in the initial active microcosms accounted for an average of around 55% of the total DCBs and 77% of the total recovered chlorinated benzenes.

The enrichments were calibrated using the water calibration since it was assumed that the inoculum would not have any significant sorptive affect. This assumption appeared to be incorrect. This is evidenced by the decrease in the initial measured DCBs levels for enrichments #1 through #3 (Figure 4.13 through Figure

4.15). (The size of the inocula increased from enrichment #1 through #3.) The MCB production in the enrichments ranged from 14% to 62% of the total DCBs and ranged from 34% to 84% of the total recovered chlorinated benzenes.

The K microcosms and the K enrichments will continue to be monitored.

5.F Louisiana Wetland Sediment

MCB production was noted in all active L microcosms. The MCB production ranged between 66% and 92% of the total DCBs and ranged from 78% to 96% of the total recovered chlorinated benzenes. While 1,2-DCB was the only DCB isomer completely consumed, the initial dechlorination of 1,3-DCB seemed to be favored over 1,2-DCB. Both 1,2- and 1,3-DCB were favored over 1,4-DCB initially. After the second feeding of DCBs (microcosms #1 and #3), this changed. From the few data available at this time, 1,4-DCB and 1,3-DCB seemed to be equally favored and 1,2-DCB the least favored.

Due to the much increased rate of dechlorination in the microcosms fed the second dose of DCBs, it is possible that the microbes responsible for the dechlorination use the DCBs as metabolic substrates.

All three DCB isomers were also dechlorinated in all microcosms not fed YE. They appeared to be dechlorinated at similar rates to those microcosms fed YE. This was probably due to a high level of electron donor naturally occurring at the site, evidenced by high CH_4 production.

The enrichments were calibrated using the water calibration since it was assumed that the inoculum would not have any significant sorptive affect. This assumption appeared to be incorrect. This is evidenced by the decrease in the initial measured DCBs levels for enrichments #1 through #3 (Figure 4.20 and Figure 4.21). (The size of the inocula increased from enrichment #1 through #3.) The MCB production in the one enrichment that has evidence of dechlorination (enrichment #3) accounted for 32% of the total DCBs and 78% of the total recovered chlorinated benzenes. In this enrichment, 1,2-DCB was the most favored DCB isomer followed by 1,3- and 1,4-DCB.

These microcosms and the enrichments will continue to be monitored.

5.G Robins Air Force Base at Well BIA4 at 17 and 25 Feet

MCB formation was observed in all active microcosms. The MCB accumulation in the 25-foot depth microcosm accounted for a range of 43% to 98% of the total DCBs and an average of 99% of the total recovered chlorinated benzenes.

The MCB accumulation in the 17-foot depth microcosms accounted for between 6% and 44% and a range of 6% to 100% of the total recovered chlorinated benzenes. The low end of that spectrum were the microcosms that were given high DCBs and were not degrading them (microcosms #2 and #3) and the high end of that

spectrum were the microcosms that were only given low levels of DCBs (microcosm #4).

The dechlorination of 1,2-DCB was favored over the dechlorination of 1,3-DCB. Both these isomers were much favored over the dechlorination of 1,4-DCB.

Due to the quick onset of MCB production and DCB consumption in all active microcosms, including those not fed YE, it is possible that DCB dechlorination is relatively active at the site.

DCB dechlorination rates increased with each subsequent DCB addition. This suggests that the microbe responsible for the dechlorination may use DCB as an energy source. From Figure 4.22, it also appears that purging the microcosms increased the rate of DCB dechlorination. This could be because the high levels of MCB slowed the dechlorination.

DCB dechlorination slowed down after the third addition of DCBs in microcosm #1 from the 25 foot depth (Figure 4.22). This decreased dechlorination rate does not appear to be caused by a lack of electron donor since there appears to be ample VFAs left in the microcosm (Figure 4.23). Another explanation could be that the MCB levels were high enough to exhibit a toxic affect. This seems to be unlikely since dechlorination rates were faster earlier in that same microcosm during periods of higher MCB levels. In addition, a needed nutrient could have been depleted and the dechlorinating population was limited. A final explanation is that the culture could have lost some of the DCB degradation ability of started to die off

during the 20 days is existed without DCBs before the fourth DCB addition.

Slight benzene production (around 1% of the total MCB recovered) was observed in microcosms #1 (from both the 17 and 25 foot depths) and #2 (from the 25 foot depth) (Figure 4.32, 4.33 and 4.44). That benzene accumulated only to low levels, could have two explanations. Perhaps benzene production rates and destruction rates were nearly equal. Several reports document the possibility of anaerobic benzene degradation under methanogenic conditions (Grbic-Galic et al., 1987; Weiner and Lovley, 1998b) and sulfate reducing conditions (Edwards et al., 1992; Lovley et al., 1995; Phelps et al., 1996; Weiner and Lovely, 1998a). An alternative explanation would be that only small amounts of benzene were produced. This second explanation seemed more likely, since the total amount of benzenes (DCBs, MCB and benzene) remained constant (with a bit of scatter) (Figures 5.1 and 5.2).

In addition, the results gathered thus far corroborate Nowak et al.'s observation that benzene production only occurred while the organism was dechlorinating a chlorinated-benzene with at least two chlorines (Figures 5.3 through 5.6) (Nowak et al., 1996). The benzene production, possibly from MCB, could be a co-metabolic process while the organism was degrading DCBs. Benzene seemed to start accumulating only when the MCB levels reached around 60 μmoles/microcosm. It could be, that this level of MCB is necessary for the kinetics of the co-metabolic transformation to become favorable. Another explanation for the slight benzene accumulation

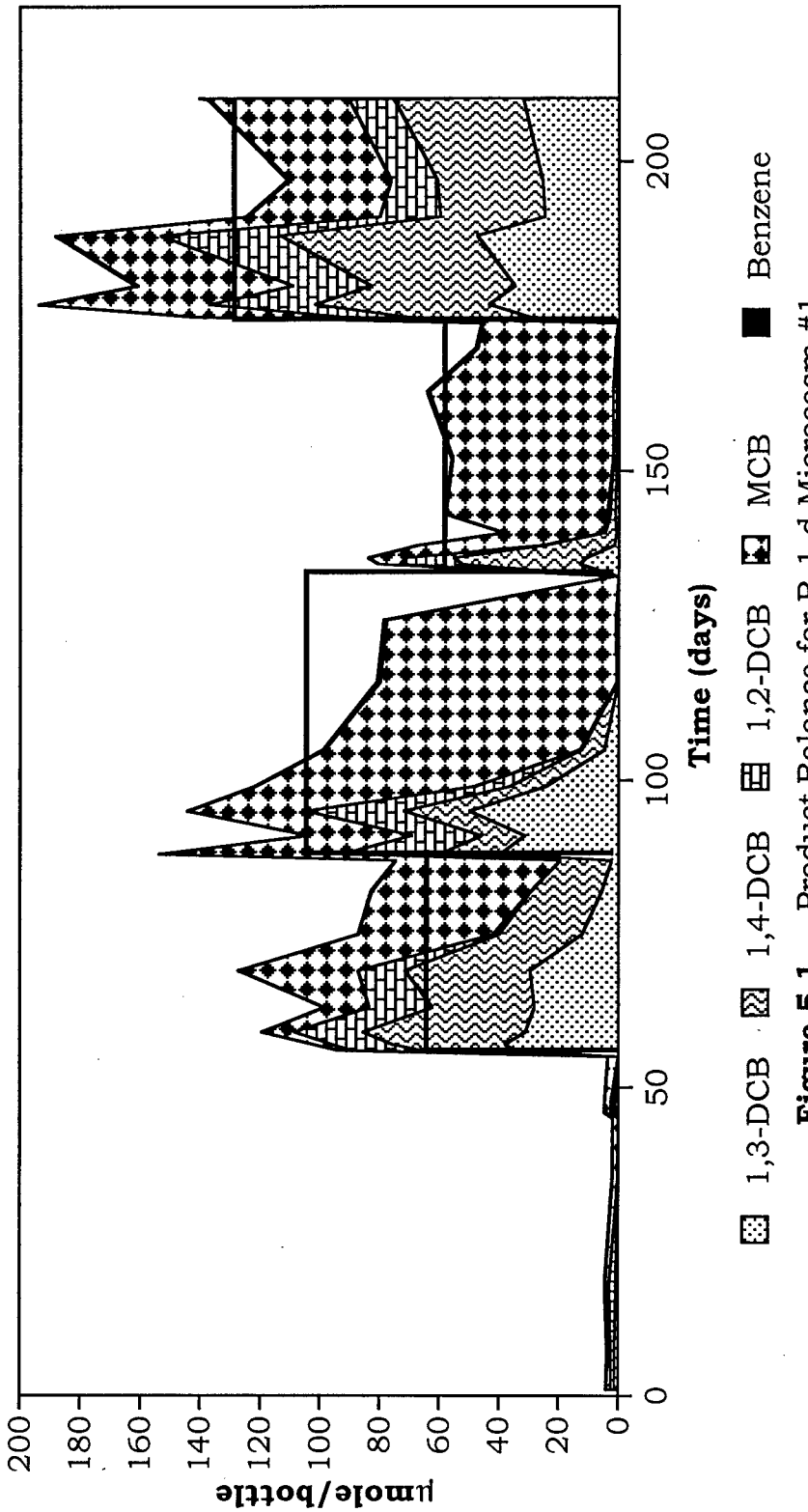


Figure 5.1 -- Product Balance for R-1-d Microcosm #1

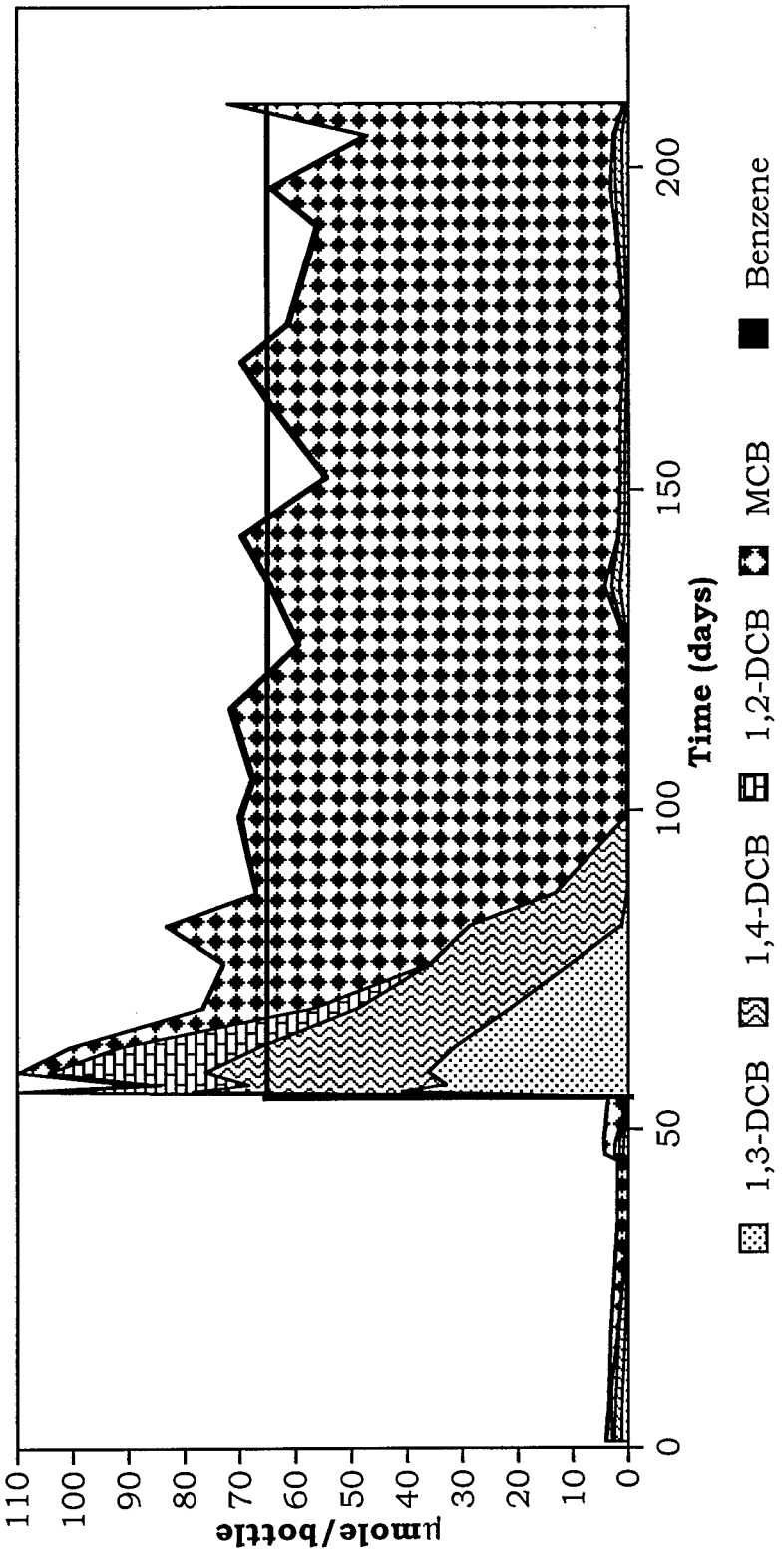


Figure 5.2 -- Product Balance for R-1-d Microcosm #2

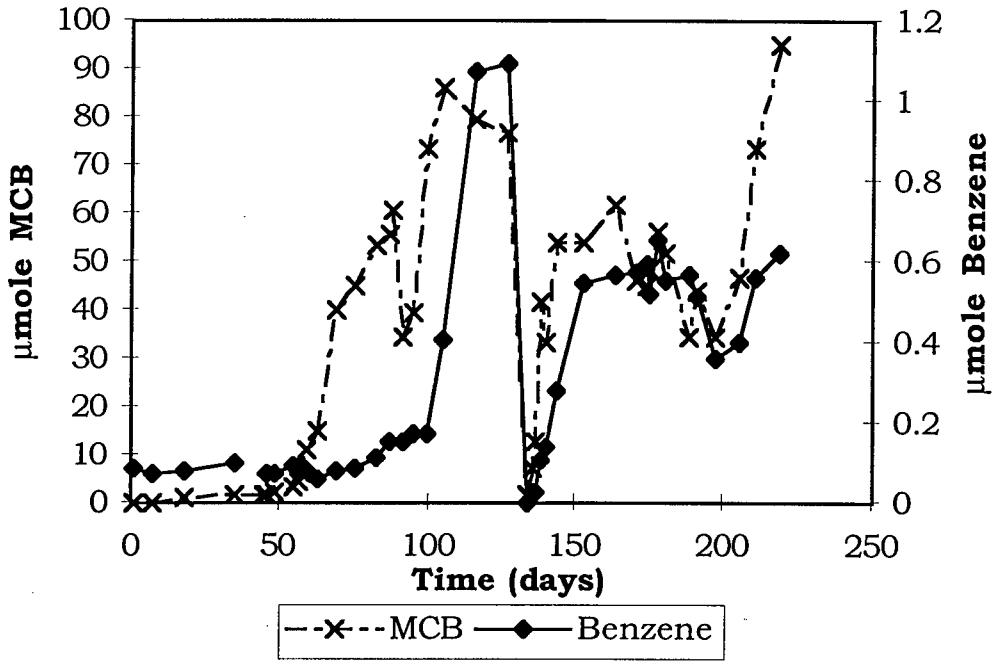


Figure 5.3 -- Benzene and MCB Concentrations in R-1-d Microcosm #1 (Fed)

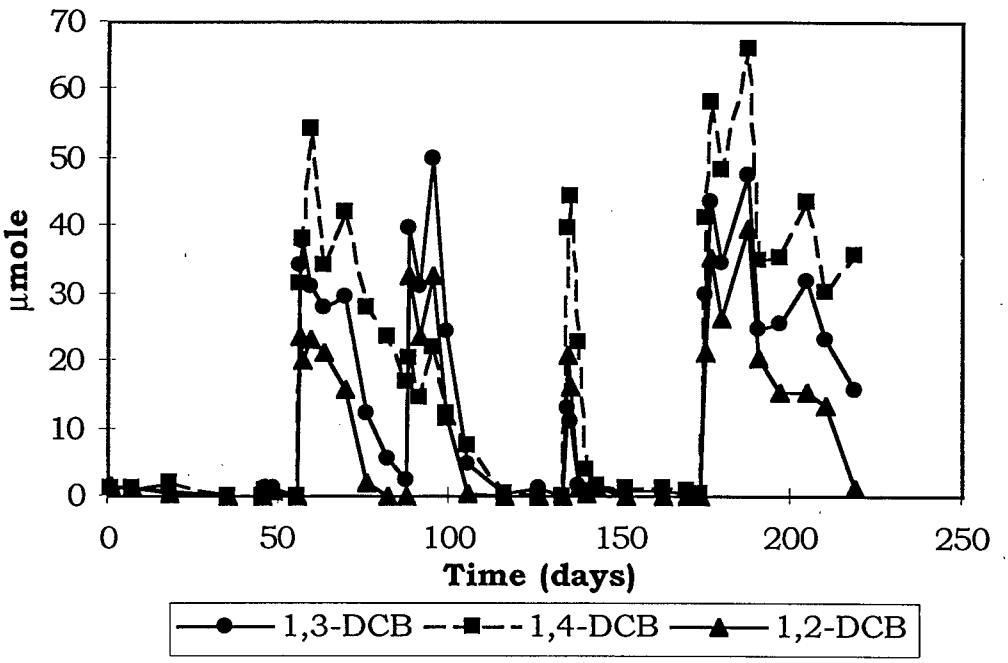


Figure 5.4 -- DCB Concentrations in R-1-d Microcosm #1 (Fed)

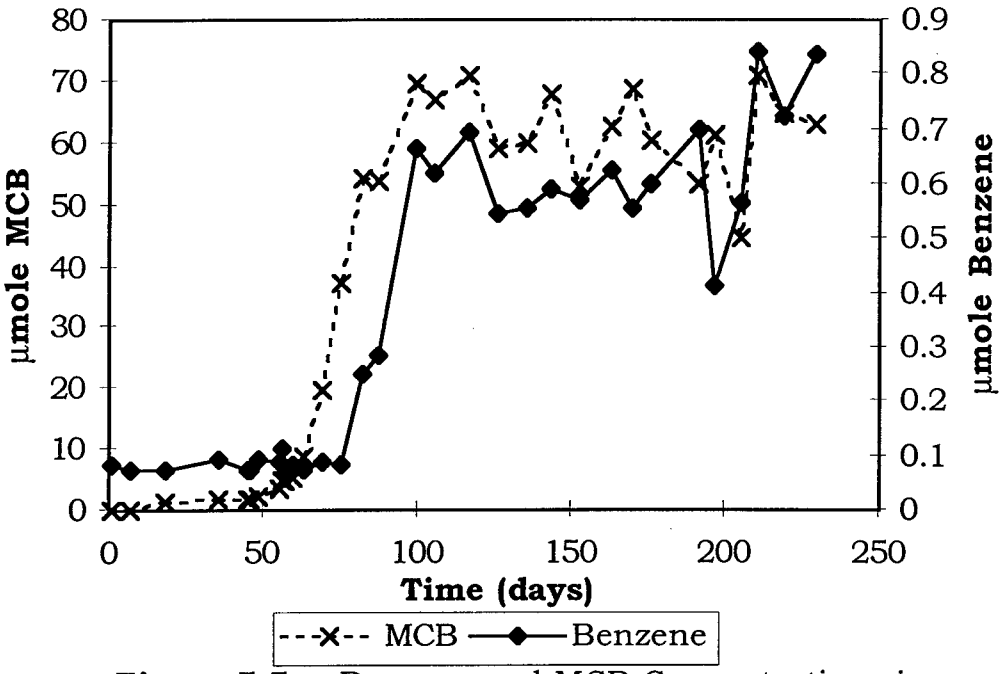


Figure 5.5 -- Benzene and MCB Concentrations in R-1-d Microcosm #2 (Fed)

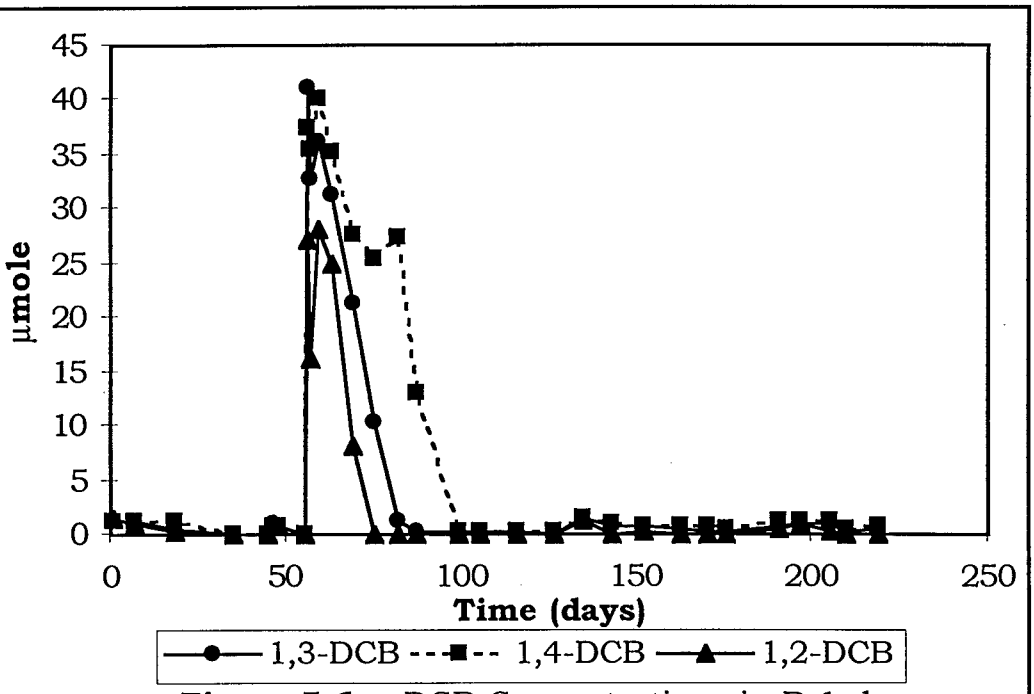


Figure 5.6 -- DCB Concentrations in R-1-d Microcosm #2 (Fed)

is that MCB may "loiter" in the dechlorinating enzyme's active site and occasionally be completely reduced to benzene.

The enrichments for the 17-foot and 25-foot depth degraded 1,2-DCB first followed by 1,3-DCB and 1,4-DCB. The MCB accumulation in microcosms from both the 17- and 25-foot depth sites accounted for between 0.7% and 28% of the total DCBs. The MCB recovered accounted for a range of 3% to 24% of the total recovered chlorinated benzenes for those enrichments given high DCBs. Enrichment #1 from the 17-foot depth had a higher percent accumulation of MCB from DCBs but this enrichment was never given high levels of DCBs.

A few R-1-s microcosms seemed to lose the ability to degrade DCBs when high levels were added (Figure 4.39, 4.40, and 4.49). In general, the R-1-s microcosms were more sensitive to the increase to high DCB levels than the R-1-d microcosms. It is possible that at the site, DCB degradation was occurring more rapidly and for a longer time at the 25-foot depth and therefore the microorganisms were more adapted to DCB degradation. It is also possible that the soil at the 25-foot depth contained a higher concentration of a needed nutrient than at the 17-foot depth.

5.H Engineering Significance

The main significance of these findings is that anaerobically, MCB will accumulate from the dechlorination of DCBs. The anaerobic production of benzene from MCB is very limited and ceases once the DCBs are gone. This can be a problem because MCB is also

a regulated chemical in groundwater with a MCL of 0.1 mg/L (Environmental Protection Agency, 1998a). Both the slight benzene accumulation and the more significant MCB accumulation could both be degraded aerobically if the plume were to migrate into an aerobic zone (Reineke and Knackmuss, 1984; Nishino et al., 1992; Gibson et al., 1968).

5.I Lessons Learned

Several lessons have been learned during the duration of this study.

(1) Soil microcosms systems containing appreciable small rocks need to be treated delicately as hairline fractures can develop and cause the microcosms to break. It is suggested to use either an orbital shaker or a "gentle" setting on a wrist-action shaker, if agitation must be employed at all (e.g. to dissolve additions of neat DCBs).

(2) The presence of a LNAPL phase can cause numerous analytical difficulties -- the greatest being the inability to obtain an accurate standard curve. The LNAPL phase can act as a sink into which the DCBs are drawn.

(3) It is very difficult to sterilize soil systems by autoclaving. It is recommended that microcosms containing a high degree of suspended matter be carefully monitored. They can be monitored by

watching for a decrease in H_2 levels, a change in CH_4 levels or a change in VFA levels.

(4) As was shown with the problems from the Kelly and Louisiana enrichments, the enrichments need individual calibrations from each inocula size to obtain accurate values for the DCB and MCB data.

(5) The soil samples need to be extremely well mixed or the microcosms can exhibit different sorptive properties which can cause quantification errors.

CHAPTER SIX -- CONCLUSIONS

Several conclusion can be drawn from the present research.

(1) DCB reductive dechlorination to MCB was observed in microcosms prepared from five of the eight studied sites (digested sludge from the Ithaca Wastewater Treatment Plant, Louisiana wetland sediment, Kelly Air Force Base, Robins Air Force Base at Well BIA4 at 17 feet and at 25 feet). The MCB production accounted for about 10% of the total DCBs added to the microcosms prepared from the treatment plant sludge, and accounted for between 6% and 40% of the total added DCBs in microcosms from Robins Air Force Base well BIA4 at 17 feet. The MCB production in all the other successful microcosms varied but accounted for between 50% to 100% of the total DCBs added, with those from the Louisiana wetland site and Robins Air Force Base at well BIA4 at 25 feet being the most successful.

(2) DCB dechlorination to MCB was possibly occurring under natural conditions and was most likely occurring on site at Robins Air Force Base at Well BIA4 at 17 and 25 feet and within the Louisiana wetland sediment. It may also have occurred in situ at Kelly Air Force base, but since the microcosms had to be perturbed from their natural state, the extent of in situ dechlorination at Kelly Air Force Base cannot be determined.

(3) Slight benzene production from DCB degradation was observed in microcosms made from sediment from Robins Air Force Base (well BIA4 at 17 and 25 feet). This is only the second reported instance. In addition, it seems that benzene production occurred only when DCBs were simultaneously dechlorinated. The benzene production accounted for about 1% of the total recovered MCB. The benzene production was most likely due to a co-metabolic transformation of MCB, coincident with DCB transformation.

(4) Generally, 1,2-DCB was the most readily degraded DCB isomer. 1,3-DCB was the next most readily degraded isomer, distantly followed by 1,4-DCB.

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